



57-60L

1960-1969







BIODEGRADATION OF HYDROCARBONS AS A REMEDIATION METHOD  
FOR PETROLEUM CONTAMINANTS IN THE ENVIRONMENT OR AS A  
TREATMENT METHOD FOR PETROLEUM WASTES  
(A Review and Analysis of Recent Field Study Literature)

Thesis  
2884

by

James Edwin Lubbers

B.S., Chemistry, Vanderbilt University, 1977

Submitted to the Department of Chemical and  
Petroleum Engineering and the Faculty of the  
Graduate School of the University of Kansas  
in Partial Fulfillment of the Requirements  
for the degree of Master of Science



## ABSTRACT

BIODEGRADATION OF HYDROCARBONS AS A REMEDIATION METHOD FOR PETROLEUM CONTAMINANTS IN THE ENVIRONMENT OR AS A TREATMENT METHOD FOR PETROLEUM WASTES (A Review and Analysis of Recent Field Study Literature)

James Edwin Lubbers, M.S.  
University of Kansas, 1989

Professor in Charge: Dr. Floyd W. Preston

## Purpose

The U. S. Navy Petroleum Office (NAVPETOFF) is developing future Navy petroleum sludge disposal and soil decontamination procedures. This project was conducted for NAVPETOFF to aid that development by evaluating the use of bacteria to eliminate petroleum hydrocarbons as a disposal or decontamination option.

## Procedure

Electronic data base searches, interviews with bioremediation researchers, and manual literature searches were conducted to collect information about microbial bioremediation from sources which postdate the 1984 amendments to RCRA. From that body of information, reports of field applications of microbial bioremediation on petroleum wastes or contaminants were set apart as the primary references for evaluation development.

Summaries of reported microbial bioremediation methods were developed and presented. These summaries are introduced by a review of the biologic limits and processes of the microbes commonly used for bioremediation. The body of these summaries describes and illustrates their techniques. Each summary concludes with an evaluation in the form of a report of the method's effectiveness.

## Conclusions

Bioremediation is not universally applicable. Where





site evaluation indicates it is feasible, it may not meet local regulatory limits. Optional remediation methods merit cost and benefit analysis. Engineering and political limits should be defined during site evaluation. For soil contamination, a critical engineering constraint is local geohydrology. If the petroleum contaminated media has low permeability to water, current bioremediation methods will have little success. Soil, contaminants, water and microbes interact in a complex, and site specific manner. Bioremediation occurs naturally but the rate of remediation may be enhanced under favorable conditions. The risk of increased toxicity from microbial metabolism of target and coincident contaminants must be understood and accommodated. Successful treatments of soil contamination *in situ* by bioaugmentation will be limited in number.

### Recommendations

The project recommends microbial bioremediation be considered one of many pollution control tools, whose skillful and successful use will require careful preparation. This preparation should be coordinated with the Federal agency responsible for developing treatment methods for petroleum contamination and petroleum wastes: the Robert S. Kerr Environmental Research Laboratory in Ada, Oklahoma.



## ACKNOWLEDGEMENTS

To Doctor Floyd Preston for his patience and encouragement.

To the staff of the U.S. Environmental Protection Agency's Robert S. Kerr Environmental Research Laboratory in Ada, Oklahoma, with special thanks to Doctor William Dunlap for scheduling key interviews there.

To the Library staff at the Environmental Protection Agency Offices in Cincinnati, OH. All were helpful, but Mr. Robert Rettig earned personal recognition by his thorough preparation for my research there and enthusiastic support during same.

To the staff of the University of Kansas Writing Center in praise of their responsiveness and endurance. I am especially grateful for the candid advice of Karen Hellekson.

This project was conducted in coordination with the United States Navy Petroleum Office (NAVPETOFF) in Cameron Station, Alexandria, Virginia and the United States Navy Postgraduate School (NPS) in Monterey, California. NAVPETOFF defined the requirements for the project and encouraged a thorough yet practical product. NPS funded my entire graduate study program, with additional financial support of the travel and miscellaneous expenses related to this project.



# TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS . . . . .	i
TABLE OF CONTENTS . . . . .	ii
LIST OF FIGURES . . . . .	iv
LIST OF TABLES . . . . .	v
SUMMARY . . . . .	vi
CHAPTER	
I. INTRODUCTION . . . . .	1
A. Purpose of Project . . . . .	1
B. Nature of Reviews . . . . .	1
C. Approach to Literature . . . . .	2
1. Methods of literature search employed . . . . .	2
2. Scope of literature on biodegradation . . . . .	5
3. Published literature . . . . .	5
4. Unpublished research . . . . .	8
5. Relation of search to U. S. Navy needs . . . . .	10
II. BACKGROUND . . . . .	12
A. Definition of Hydrocarbon Biodegradation . . . . .	12
B. Relation of Biodegradation Research to Environmental Concerns . . . . .	12
1. Responses to environmental concerns	12
2. Catalysts to concern and research .	13
3. Options to address environmental concerns . . . . .	13
4. The option of bioremediation . . .	14
5. Priority of efforts . . . . .	15
C. Basic Concepts . . . . .	16
1. Liebig's Law of the Minimum . . . .	17
2. Shelford's Law of Tolerance . . . .	17
D. Specific Biodegradation Processes . .	18
1. Fermentation . . . . .	19
2. Anaerobic respiration . . . . .	19





3. Aerobic respiration . . . . .	21
E. Summary of Biometabolism Review . . .	22
III. BENEFITS OF BIOREMEDIATION . . . . .	24
IV. ENVIRONMENTAL RISKS FROM BIOREMEDIATION .	25
V. TREATMENT METHODS REPORTED . . . . .	27
A. Classification of Methods . . . . .	27
B. Removal and Treatment . . . . .	28
1. Pump-out and treatment of groundwater . . . . .	28
2. Removal of contaminated medium and treatment at a distance from the contaminated site . . . . .	47
C. <i>In situ</i> Treatments . . . . .	60
1. Description of process . . . . .	60
2. Examples of process . . . . .	63
3. Evaluation of process . . . . .	71
VI. COMMON ELEMENTS OF SUCCESSFUL TREATMENTS .	73
A. Elements common to all bioremediation methods . . . . .	73
B. Common elements to bioremediation methods for petroleum contaminants/ wastes . . . . .	73
1. Product recovery . . . . .	73
2. Site characterization . . . . .	74
3. Monitoring, maintenance and modification . . . . .	83
VII. ERRORS OF PROCESS OR TREATMENT . . . . .	84
A. Ineffective Bioaugmentation . . . . .	84
B. Incomplete Soil Geohydrology Characterization . . . . .	85
C. Failure to Monitor/Modify Applied Methods . . . . .	86
D. Failure to Coordinate with the Robert S. Kerr Environmental Research Laboratory	88
VIII. CONCLUSIONS . . . . .	90
IX. RECOMMENDATIONS . . . . .	93
REFERENCES . . . . .	94
APPENDIX A . . . . .	A-1



## LIST OF FIGURES

Figure	Page
1. Schematic of the equipment and flow pattern in a fixed film bioreactor . . . . .	31
2. Schematic of the material flow in an activated sludge system . . . . .	37
3. Flow diagram/schematic of Liquid/Solids Tank Bioreactor . . . . .	50
4. Simplified cross sectional view of an <i>In situ</i> treatment process . . . . .	61





## LIST OF TABLES

Table	Page
1. Petroleum Hydrocarbon Contamination of Groundwater Treated by Groundwater Extraction and Bioremediation . . . . .	42
2. Petroleum Hydrocarbon Contamination of Soils or Petroleum Sludge Volume Remediation by Extraction and Biotreatment . . . . .	57
3. <i>In situ</i> Bioremediation of Contaminated Soils and Aquifers (Independently or in combination combination with other process) . . . . .	64



BIODEGRADATION OF HYDROCARBONS AS A REMEDIATION METHOD  
FOR PETROLEUM CONTAMINANTS IN THE ENVIRONMENT OR AS A  
TREATMENT METHOD FOR PETROLEUM WASTES (A Review and  
Analysis of Recent Field Study Literature)

SUMMARY

The U. S. Navy Petroleum Office (NAVPETOFF) is developing future Navy petroleum sludge disposal and soil decontamination procedures. This project was conducted for NAVPETOFF to aid that development by evaluating the use of bacteria to eliminate petroleum hydrocarbons as a disposal or decontamination option.

Electronic data base searches, interviews with bioremediation researchers, and manual literature searches were conducted to collect information about microbial bioremediation from sources which postdate the 1984 amendments to RCRA. From that body of information, reports of field applications of microbial bioremediation on petroleum wastes or contaminants were set apart as the primary references for evaluation development.

Summaries of reported microbial bioremediation methods were developed and presented. These summaries are introduced by a review of the biologic limits and processes of the microbes commonly used for bioremediation. The body of these summaries describes



and illustrates their techniques. Each summary concludes with an evaluation in the form of a report of the method's effectiveness.

Microbial bioremediation methods reported in recent literature are of two general classes. They are labelled by this project as Remove and Treat methods or *In situ* methods. Remove and Treat methods involve transfer of contaminated material to a site or an equipment array for treatment. These methods are of two subclasses which involve either pump-out and treatment of contaminated ground water or relocation and treatment of wastes or contaminated soil. By contrast, *in situ* methods eliminate contamination where discovered.

For all three methods, this project presents summaries of technique and treatment examples for both petroleum wastes and contaminated soils. For treatments of sludge by microbial biodegradation, relocating these materials to a structure or area developed for that purpose is the most common method. Contaminated soil treatments include examples from all three methods.

Available information on the subject of microbial bioremediation of petroleum wastes or contamination indicates it is an effective cleanup technology where its





use is feasible. While many limitations to the rate of cleanup may be overcome, if the petroleum contaminated material is not permeable, microbial bioremediation will be ineffective. If naturally occurring bacteria colonies capable of degrading petroleum contaminants *in situ* are not available, microbial bioremediation will be very difficult.

NAVPETOFF requested special attention be given to reporting the state of the art and apparent merit of using specialized bacteria for bioremediation of petroleum wastes or soil contamination. Literature on this subject calls this process bioaugmentation. It involves adding bacteria into the waste or contaminated site, which bacteria are known to degrade the target contaminant. Information available at this writing indicates bioaugmentation has limited effectiveness in applications outside of carefully constructed and controlled treatment containers. No single or small range of bacteria species can reduce the complex hydrocarbon molecules of petroleum to environmentally safe materials. Many different species interact in this process. Modern biotechnology cannot produce sufficiently diverse bacteria colonies capable of fully



degrading the wide variety of hydrocarbons in petroleum. Furthermore, bacteria currently used for bioaugmentation do not survive very long in the natural environment. Bioaugmentation will seldom be a cost effective option for treatment of Navy petroleum waste or contamination problems.

This project provides NAVPETOFF with a single reference which collates information about petroleum hydrocarbon microbial bioremediation from the much larger set of reports about organic chemical remediation and bioremediation. The specific conclusions of this project emphasize the site specificity of microbial bioremediation's potential. They also stress the need for careful evaluation of its risks and benefits before and during application.

I recommend microbial bioremediation be considered one of many pollution control tools, whose skillful and successful use will require careful preparation. This preparation should be coordinated with the Federal agency responsible for developing treatment methods for petroleum contamination and petroleum wastes: the Robert S. Kerr Environmental Research Laboratory in Ada, Oklahoma.





## I. INTRODUCTION

### I-A. Purpose of Project

This project involves a review and analysis of recently published literature on the use of microbes to treat petroleum wastes or clean up petroleum spills on land. This project responds to a request from the U. S. Navy Petroleum Office for a survey of literature on this subject. Information from this project will be used to "formulate future Navy petroleum sludge disposal and soil decontamination procedures" (Carstanjan, 1989). Promising methods reported by this project may be subjected to Navy field tests.

### I-B. Nature of Reviews

Each item of literature reviewed was examined for information to answer the following questions.

- Does the document report biological treatment methods which U. S. Navy could use to solve environmental problems resulting from the storage and transportation of bulk quantities of refined petroleum products? If so, what are these methods?



- Are there common elements in reports of successful biological treatments which the Navy should include when using this process?

- What errors or failures are reported by the literature that may be avoided by the Navy when using microbes to treat petroleum wastes or clean up petroleum spills?

- Where are the sources of information or assistance in this field which are available to the Navy?

#### I-C. Approach to Literature

##### I-C-1. Methods of literature search employed

The methods, which revealed literature holding answers to the questions above, fall into four categories. These categories are summarized below in decreasing order of the volume of references they produced.

##### I-C-1-a. Electronic data base searches

A subject search of the National Technical Information System's Silver Platter data base was conducted for me by librarians at the U. S. Environmental Protection Agency's Cincinnati, Ohio Research Offices. The Silver Platter data base contains identification data and abstracts of U. S. government sponsored research documents. Publication subjects, and abstract texts were searched



for key words such as "bioremediation," (using microbes to clean up environmental contamination) "biodeterioration," "oil pollution," "bacteria," and "microorganisms". These searches used such terms singly and in combination with each other. Candidate literature identified by this process was provided to me during a research visit to that library. I reviewed that body of literature, and those found by my own search of the Silver Platter Database conducted there, to determine how well any given document held answers to the questions of section I-B.

#### I-C-1-b. Interviews

During interviews with research personnel or business operators in the field of bioremediation, I asked them to identify publications they thought would be useful in my research. They often provided me with copies of candidate publications directly, from their own files. They were also able to direct me to sources of these publications. I reviewed candidate publications identified by this process to determine the degree to which they held answers to the questions of section I-B.

#### I-C-1-c. "Daisy Chain" reference searches

When an item of literature related to bioremediation of petroleum contamination in the environment was receiv-



ed, I would carefully review the references of that document and attempt to gain access to them. On finding useful reports among those references, I would in turn pursue their references. I would continue this process until I could eliminate a candidate because it did not answer the questions reported above, I came full circle to references I already held, or I determined it was a new candidate.

#### I-C-1-d. Publication searches

Publications such as Pollution Abstracts, Index to Scientific and Technical Proceedings and Science Citations Index were manually searched by subject category. Subjects searched were those from their subject sets which best matched the terms that efficiently produced references during the electronic searches discussed above. Current periodicals, known through one of the previous methods to contain articles or information about bioremediation, were manually searched for candidate literature not yet catalogued by the Silver Platter database or other abstract collections. Candidate reports found by these manual processes were examined as before to determine if they answered the questions of section I-B.





## I-C-2. Scope of literature on biodegradation

The number of publications about the interactions of petroleum and microbiota revealed by the methods of literature search described above was very large, and decades in vintage (Atlas, 1981). Entire textbooks have been prepared on the subject, whose references to literature number in the hundreds (Atlas, 1984). The National Technical Information Service (NTIS) produces bibliographies which catalog hundreds of published works in this field (NTIS 1988a, NTIS 1988b). One of these bibliographies, for example, lists 173 documents published in the period January 1970 to January 1988 on the subject of "Biodeterioration of Oil Spills" .

## I-C-3. Published literature

To maximize the practical utility of this report, I imposed the following conditions on published literature discovered by searches reported above.

- The document must report a site demonstration or field application of bioremediation. The target of the remediation must have been petroleum wastes/sludges or soil/aquifer contamination by one or more components of the refined petroleum products received, stored and distributed at U. S. Navy bulk petroleum terminals.



- The publication year of documents which meet the above condition must not be earlier than 1984.

Exceptions were made where a publication was widely cited in recent literature as fundamental to a specific area or contained useful summaries of practical efforts in the field (Atlas, 1981; Raymond et al., 1976).

These limits served two important purposes:

- First, since petroleum hydrocarbons are a subset of a wide variety of organic chemicals subjected to bioremediation studies, focusing on field studies of petroleum biodegradation minimizes the need to extrapolate techniques or results to Navy problems. There are many differences between any two waste accumulations or spill sites. Minimizing the difference between Navy situations and those reported in the literature increases the likelihood that lessons learned from treatment or research will be useful to the Navy.

- Second, the selection conditions focused attention on current developments in this swiftly changing field. As reported by Clinton Hall, Director of the U. S. Environmental Protection Agency's Robert S. Kerr Environmental Research Laboratory in his keynote speech to the Fifth Annual Symposium of the National Well Water



Association in May of 1985, research in the field of bioremediation was inefficient and uncoordinated before calendar year 1985 (Hall, 1985). It was the view of prominent researchers and staff at the Robert S. Kerr Environmental Research Labs that except for the Raymond/Suntech work (Raymond, 1974), notable advancements in the field of organic contaminant bioremediation do not predate the 1984 amendments to RCRA (Dunlap, 1989; Hutchins, 1989; McNabb, 1989). I found this opinion to be an underlying assumption in much of the literature on this subject. It is the basic assumption, for example, of the report of Gosse et al. to the Solid Waste Management Branch of the Environmental Protection agency (Gosse et al., 1985). I have therefore developed this condition in coordination with and as result of recommendations from experienced and influential researchers in this field. Their opinions were independently verified by publications they did not influence.

When these conditions were imposed on candidate literature, the number of documents which became subject to this project was reduced to about 100. These documents are the core of the citations of this report. Where a published reference is cited, it emerged from the



methods for searching discussed above, and survived the acceptability filter just reported.

#### I-C-4. Unpublished research

Most of the newest information about bioremediation is unpublished. Research projects about or applications of bioremediation which have no public documentation are generally of one of two types: Never/unlikely to be published, or Not yet published.

A brief discussion of these two types of unpublished research will be provided in the next two subsections of this report.

##### I-C-4-a. Never/unlikely to be published

There are bioremediation projects which will not be documented. This is particularly true of industrial applications. As suggested by various witnesses before a U. S. House of Representatives' committee (Anon., 1988a), industries are applying the benefits of this technology without fanfare or documentation for at least two reasons.

- First, since any publicity about environmental pollution from an industry is considered bad publicity, firms want to reduce or solve their environmental problems before discovery by the public or, worse, by regu-





latory agencies. The latter might impose fines or compliance costs through additional administrative burdens of inspections and reports (Anon., 1988a; Smith, 1989; Trickett, 1989).

- Second, where industries have discovered successful techniques to solve environmental problems by biotechnology, they do not wish to share this success with their competitors (Smith, 1989; Wetzel, 1987). Indeed, pointedly identified as out of bounds to my interview with their Operations Manager were details of how DETOX Industries builds the biologic consortia it uses to clean up contaminated water (Galaska, 1989).

The absence of published reports from industrial applications does limit the completeness of this document. As will be shown, however, certain basic elements of treatment technology are common to bioremediation efforts. Bioremediation research has identified or improved methods which were exported to field applications and became common elements of treatment processes. As discussed below, I interviewed several scientists and engineers active in research to discover or refine bioremediation techniques. They had worked with or knew about commercial bioremediation. Their knowledge of the field as captured by these



interviews filled some of the gaps in published literature about industrial use of bioremediation.

I-C-4-b. Not yet published

During this research, I found that new and often times promising technology is not yet documented. Developments in technology which are occurring and being implemented in 1989, such as aerobic denitrification, were dismissed as impossible three or four years ago (Dunlap, 1989; Hutchins, 1989; Hutchins and Wilson, 1989; Kuhn et al., 1988; Major et al., 1988). Although I am unable to cite printed references about this type of research, I have compensated for this limitation as follows. I have included information from interviews with representatives of industries who already use bioremediation in their business endeavors and seek new and better uses of it (Galaska, 1989; Trickett; 1989). To gather information about current but unpublished research, I interviewed research personnel at the leading edges of technology development in this field. I have included and cited information from these interviews where appropriate (Dunlap, 1989; Glaser, 1989; Hains, 1989; Hutchins, 1989; McNabb, 1989).

I-C-5. Relation of search to U. S. Navy needs



Development of the conditions which limit source documents for this review and analysis was coordinated with the U. S. Navy Petroleum Office during plan of action conferences in July and August 1989 (Schmokel, 1989). During those conferences, a Navy representative endorsed these conditions as serving the letter and spirit of the call for this literature search (Schmokel, 1989; Carstanjan, 1989). In August 1989 the U. S. Navy Petroleum Office was provided with a summary and the recommendations of this document. The limiting conditions discussed above were reexamined at that time; full approval of these conditions and my approach to literature on this subject was provided (Schmokel, 1989).



## II. BACKGROUND

### II-A. Definition of Hydrocarbon Biodegradation

Many hydrocarbon components of refined petroleum products have been well-documented as potential sources of biological activity for a wide variety microbes (Atlas, 1981; Britton, 1989; Chapelle and Morris, 1988; Galaska et al., 1989; Lee and Levy, 1989; Swindoll, 1988; Wetzel et al., 1987). A major factor in the fate of petroleum hydrocarbons released into the environment is their consumption or breakdown by microbes (Atlas, 1988; Major et al., 1989; Stover, 1989). Microbial biodegradation of hydrocarbons, then, is the biochemical reduction of their complex molecules to simpler molecules as microbes use them in support of life processes.

### II-B. Relation of Biodegradation Research to Environmental Concerns

#### II-B-1. Responses to environmental concerns

A general increase in efforts to solve environmental problems caused by organic chemical contamination has occurred in the past decade. These efforts have employed a variety of methods, biodegradation of contaminants among them. Studies of bioremediation as a clean-up method for





the environmental problems caused by extraction, transport, refinement and use of petroleum is well-dispersed in this larger body of effort.

#### II-B-2. Catalysts to concern and research

The recent increase in research and publications on the subject of remediation of environmental pollution stems largely from incentives introduced by environmental laws and regulations. Specific among the laws which spur remediation research in the United States are the Comprehensive Environmental Response, Compensations and Liability Act (CERCLA) of 1980, the Resource Conservation and Recovery Act (RCRA) as amended in 1984, and analogous legislation at state levels. These laws and regulations mandate monitoring and elimination of sources of ground water contamination (Bowlen and Kosson, 1988; Chowdhury, 1986; Harris, 1987; Leach et al., 1988; Offutt et al., 1988; Trickett, 1989; Wilson, J. T. et al., 1988).

#### II-B-3. Options to address environmental concerns

Many methods are available to control or reduce environmental contamination. Waste minimization, material recovery, or process modification are methods of avoiding contaminant or waste problems entirely. Clean up or remediation options, in addition to bioremediation, include



incineration, wet air oxidation, landfill, *in situ* fixation (vitrification), or *in situ* volatilization and recovery .

Of the various remediation methods, many local enforcement officials have yet to accept bioremediation of petroleum contaminants as a demonstrated technology. They are unaware of its success and merit, and are unwilling to approve its use. This may effectively eliminate biotreatment as an option regardless of its engineering, economic, or technical merit (Yaniga et al., 1985). An alternative cleanup process may be politically imposed. Some evidence shows this trend to be reversing however. For example, *in situ* bioremediation of a gasoline spill in California has recently been declared successful and complete (Anon., 1988b). In another situation, the Sugar Creek Missouri Refinery sludge clean-up project has been approved for bioremediation. This project is especially noteworthy in this regard since it is designated a RCRA site (Anon., 1988c; Anon., 1989b; Shepard, 1989).

#### II-B-4. The option of bioremediation

One way of solving the environmental problems created by spills, leaks, or petroleum waste products is bioremediation. Publications which reflect attempts to capitalize on and apply the hydrocarbon degrading (hydro-



carbonoclastic) capability of microbes, rather than simply characterize or report this ability, have increased as successful applications of this biotechnology have begun to occur. The increase in the number of these publications is most notable in the last 5 years (Anon., 1988a; Baker et al., 1988; Hurlburt, 1987; Keely et al., 1987; Rifai, 1988; Shepard, 1989; Thomas, et al., 1987a; Wilson, J. T. and Ward, 1987; Wilson, J. T. et al., 1986).

#### II-B-5. Priority of efforts

An important overview factor emerges from an examination of recent literature about biodegradation of petroleum wastes or bioremediation of petroleum hydrocarbon contaminants in the environment. This factor is the intense focus on treatment of contaminated water, particularly of contaminated ground water.

Petroleum waste accumulations or contamination which did not reach surface waters or aquifers because they were fixed in the unsaturated zone of the soil, or were kept out of contact with these waters, generally received little attention in research. This is so for a variety of reasons. Preeminent among them is the perceived low threat to public health from such situations (Hurlburt, 1987; Trickett, 1989).



There is another reason why petroleum wastes or spills which did not impact aquifers received little attention historically. This second reason is the limit on the authority of agencies which enforce environmental law. The jurisdiction of the U. S. Environmental Protection Agency (EPA) is limited to surface waters, outside the boundaries of an installation (public waters) and to pollution in the atmosphere. (These limits are superseded if the contaminated area is identified as a RCRA or Superfund site). The various state agencies with primacy of enforcement of environmental legislation have the same limit as described for the EPA, except their jurisdiction often includes ground water beneath an installation, when that ground water communicates with surface waters external to the installation boundaries. These jurisdiction limits focus regulatory attention on air and water pollution.

## II-C. Basic Concepts

A brief introduction to the biochemistry by which microbes reduce the complexity of hydrocarbons in petroleum will help the reader understand the various treatment methods reported later. Such an introduction will be presented shortly through summaries of germane literature. Before beginning the presentation of these summaries,





however, two basic principles of biology will be reviewed. These principles guide the design of bioremediation systems.

#### II-C-1. Liebig's Law of the Minimum

Liebig's Law of the Minimum states, "the total yield or biomass of any organism will be determined by the nutrient present in the lowest (minimum) concentration in relation to the requirements of that organism" (Atlas and Bartha, 1987). To relate this to microbes, this means that from any given set of nutrients, some item or other they require for life will run out first. As that item begins to run out, it will limit their further vitality and propagation.

#### II-C-2. Shelford's Law of Tolerance

Atlas and Bartha (Atlas and Bartha, 1987) interpret this law to state:

the abundance of organisms in an ecosystem requires a complex set of conditions . . . For an organism to succeed in a given environment, each of these conditions must remain within the tolerance range of that organism and if any condition . . . exceeds the maximum or minimum tolerance of the organism, the organism will cease to thrive and will be eliminated.

Thus, the presence of nutrients in adequate quantities will not guarantee the life and vigor of a bacteria colony. Starvation is not the only way a bacteria colony can die.



Many other environmental events or conditions control their survival and vitality.

#### II-D. Specific Biodegradation Processes

With these two basic principles of biology in mind, let us examine the biochemistry of microbial biodegradation of petroleum hydrocarbons. Microbial biodegradation of organic molecules generally proceeds by one of three biologic pathways. These are classified as processes of:

- Fermentation
- Anaerobic respiration
- Aerobic respiration.

Petroleum-degrading microbes which exhibit these processes are most often those which use organic molecules both as an energy source and as a source of carbon for their cellular structure. These double action microbes are called heterotrophic (Stover, 1989). In the following paragraphs, a brief description of the three biologic processes by which heterotrophic bacteria metabolize organic molecules (to include petroleum hydrocarbon) will be presented. An assessment will be provided of the relative importance of each of these processes in currently documented bioremediation of environmental problems related to the petroleum industry.



#### II-D-1. Fermentation

When a microbe metabolizes an organic molecule by fermentation, it benefits from a series of enzyme-mediated reactions which do not involve an electron transport chain. The enzymes which react with organic molecules, to the benefit of the microbe, are secreted, excreted, or provided by it for this purpose. Research on the use of fermenting microbes to degrade hydrocarbons common to petroleum is very new. Some work has begun to determine how best to use certain white rot fungi which can secrete enzymes known to perform the initial oxidation of complex organic components in wood preservation wastes. Other than this work, fermentation has had very little application to the problems of waste remediation. I found no documented applications of fermenting heterotrophs to the problem of petroleum-contaminated soils. Understanding and application of this type of heterotrophic bacteria is truly at its genesis (Glaser, 1988; Glaser, 1989; Glaser et al., 1989; Stover, 1989).

#### II-D-2. Anaerobic respiration

When a microbe metabolizes an organic molecule by anaerobic respiration, "it breaks down the carbon and energy source by a series of enzyme-mediated reactions in



which sulfates, nitrates and carbon dioxide serve as the external electron acceptors" (Stover, 1989). Although there have been some limited applications of anaerobic heterotrophs to petroleum wastes and land spills, the use of this type of bacteria is rare outside of certain special waste water treatment plants (Lee and Ward, 1985; Thomas et al., 1987a). As recently as 1988, in the context of discussing complete reduction of organic contaminants to carbon dioxide and water, Kuhn et al. (Kuhn et al., 1988) reported

metabolism of [benzene, toluene and xylene] in the absence of molecular oxygen has never been demonstrated under pure culture conditions, and only data from field studies in polluted aquifers suggest that a slow degradation may be possible.

Atlas states "the question of whether anaerobic hydrocarbon metabolism occurs has been quite controversial" (Atlas, 1988). He goes on to list some work from the 1960s and early 1970s which reported some anaerobic biotransformation of molecular structure for organic chemicals. Complete degradation of petroleum hydrocarbons by solely anaerobic processes is not documented. Mayfield (Mayfield, 1989) expresses reservations about the type of studies listed by Atlas: "one compound/one organism", and suggests they may not reveal what is happening in nature. Mayfield further





endorses Kuhn et al.'s summary of field studies about anaerobic bioremediation when he states "Field studies with adequate controls are sparse." It is easier to find denouncements or dismissals of the use of purely anaerobic microbes for bioremediation of organic contaminants than endorsements of or documented research about using them (Anon., 1985; Atlas, 1988; Britton, 1989; Hurlburt, 1987; Lee et al., 1988; Thomas et al., 1987a; Wilson, B. H. et al., 1986b; Wilson, J. T. et al., 1988). In some recent and current research, interest centers around the use of nitrate as an electron acceptor in a process called aerobic denitrification. This technique attempts to combine the service of anaerobic microbes which use nitrate as an electron receptor, and truly aerobic bacteria, to gain benefit from both (Bowlen and Kosson, 1988; Britton, 1988; Grbic-Galic and Vogel, 1987; Hutchins, 1989; Hutchins et al., 1989; Reinhard, 1984; Major et al., 1988; Zeyer et al., 1986).

#### II-D-3. Aerobic respiration

When a microbe metabolizes an organic molecule by aerobic respiration, oxygen is the electron receptor in enzyme-controlled reactions which change the molecule from its initial state to a simpler form. Carbon not used for



cell structure will eventually be released as carbon dioxide (Atlas and Bartha, 1987; Grubbs and Molnaa, 1988; Lee, 1989; Stover, 1989). Aerobic heterotrophs are the most widely used bacteria for bioremediation of petroleum hydrocarbon problems. Study of what best nurtures their metabolism of petroleum has been extensive (Anon., 1985; Chowdhury, 1986; Galaska et al., 1989; Hater, 1988; Lee, 1989; Matson, 1985; Spain et al., 1989; Thomas and Ward, 1989; Wetzel, 1987; Wilson, J. T. et al., 1986). For all practical purposes, when discussing the subject of bioremediation as a method to control petroleum wastes/sludges or as a process to clean up petroleum spills, aerobic microbes may be understood to comprise the active degraders (Hurlburt, 1987; Smith and Collins, 1984; Stetzenbach, 1986).

#### II-E. Summary of Biometabolism Review

No one bacteria species does the entire job of breaking down a complex organic molecule to environmentally safe material. Groups of bacteria work together. Each species has its own role in a process of many individual steps. Their combined efforts are required for successful treatments (Dunlap, 1989; Field et al., 1988; Glaser, 1989; Hains, 1989; Kuhn et al., 1988). Whatever the process used



by the degrading microbes to reduce the complexity of petroleum hydrocarbons, many different kinds of nutrients will be required. A properly designed bioremediation system will cause the contaminate which the treatment is intended to remove to be the limiting nutrient (Chowdhury, 1986; Galaska, 1989; Galaska et al., 1989; Hains, 1989; Hutchins, 1989). Whether expressed or not, the intent of the design of bioremediation systems for cleanup of petroleum-contaminated media is twofold:

- Manipulation of the immediate environment of the degrading organisms in accordance with Liebig's Law of the Minimum.

- Maintenance of the environment of the degrading organisms within their tolerances to temperature, salinity, pH and other features of their physical or chemical surroundings in accordance with Shelford's Law of Tolerance.

If petroleum hydrocarbons are available nutrients, other nutrient requirements are met, and environmental factors are within tolerance, hydrocarbonoclastic bacteria can use those hydrocarbons for food.



### III. BENEFITS OF BIOREMEDIATION

The attractiveness of applying bioremediation technology to problems of petroleum pollution in soil and water stems from two sources.

- First, its successful application can yield destruction of petroleum pollution to the limits of detection. In the ideal case bioremediation of petroleum-contaminated media produces carbon dioxide ( $\text{CO}_2$ ), water, clean media and environmentally safe biomass (Atlas, 1981; Field et al., 1988; Galaska et al., 1989; Kuhn, 1988; Lee, 1989; Lee et al., 1988; Wilson, J. T. et al., 1988).

- Second, a strong driving force behind the popularity of this successful method, is the historically low cost of bioremediation of petroleum-contaminated media (Harris, 1987). The cost of bioremediation is variously quoted as 8-25% that of incineration, 15-50% that of in place fixation, 18-60% that of off-site land fill (Anon., 1988a; Grubbs and Molnaa, 1988; Lee et al., 1988), and 30-60% that of air strippers or carbon adsorption treatments for removal of volatiles (Chowdhury, 1986; Lee et al., 1988).





#### IV. ENVIRONMENTAL RISKS FROM BIOREMEDIATION

For certain chemicals which microbes can use as food sources (substrates), harmful byproducts may result as these chemicals are metabolized by the organisms. Some biodegradation products are more toxic than is the original contaminant. The classic example of this problem is the contaminant trichloroethylene. Bioremediation of it commonly produces the more toxic vinyl chloride as a product (Torpy et al., 1989; Wilson, B. H. et al., 1986c). Certain other halogenated hydrocarbons, in use as pesticides, are known to produce less chemically complex but more toxic compounds when biodegraded (Konieczny et al., 1985). Literature in this area suggests this problem is not encountered if the only candidates for bioremediation are petroleum hydrocarbons. In the field however, contaminated sites and media are commonly a mixture of fuels, solvents and waste materials. At the site of Navy fuel spills or petroleum sludge accumulations, fuel additives and metal contaminants will commonly reside along with petroleum hydrocarbons in the contaminated zone/material. There may be risk of increased toxicity in the treatment zone through biodegradation of these other contaminants.



This risk must be systematically evaluated, fully understood and appropriately mitigated.



## V. TREATMENT METHODS REPORTED

### V-A. Classification of Methods

Methods of environmental cleanup of petroleum pollution by microbial action may be conveniently divided into two classes.

- Removal of the contaminated medium and treatment at a distance from the contaminated site: Removal and treatment.

This class of treatment techniques is conveniently divided into two sub-classes:

- Pump-out and treatment of ground water.
- Relocation and treatment of petroleum-contaminated soils/sludges.

- Treatment of the contaminated medium in place with little or no movement or transportation of the polluted material: referenced in most literature by the Latin: *in situ*. *In situ* treatments may be applied alone or in combination with other processes.

This chapter presents these classes of treatment techniques in the following sequence: the two Remove and treat method sub-classes will be presented first, followed by a presentation of *in situ* methods. For each method, an



initial section describes its techniques. Following this description, tables of examples are presented, one each for the two sub-classes of Remove and treat techniques, and one for *in situ* treatments. Immediately after each table will be an evaluation of the importance or utility of the method.

#### V-B. Removal and Treatment

##### V-B-1. Pump-out and treatment of ground water

##### V-B-1-a. Description of process

Petroleum products can spread outward and downward into soil, through the soil's upper layers, toward or until they reach an aquifer. If they reach an aquifer, they may then travel more quickly on or with its fluids to places where the health or quality of life of inhabitants of the environment will be degraded by their effect (Barker, et al., 1987; Mackay and Cherry, 1989; Reinhard et al., 1984; Shepard, 1989; Spain, 1989; Wilson, B. H. et al., 1986a; Wilson, B. H. et al., 1986b; Wilson, J. T. and Ward, 1987). Pump-out and treatment of ground water attempts to reverse this process. Water is taken out of the ground and contaminants removed. The water is then discharged off-site or reintroduced within the contaminated site.





Of the multitude of chemicals in petroleum products which could be transported in or with ground water in aquifers, aromatic hydrocarbons have received disproportionate attention as producing risks to human health. Spills on or into soil of lighter grades of refined petroleum fuels are most likely to cause contamination of aquifer water with aromatic hydrocarbons. Although comprising one-half or less by weight of common refined fuels, such as unleaded gasoline, kerosene, or No. 2 Fuel Oil, aromatic hydrocarbons comprise more than 93% of the contaminants in water-soluble fractions of these fuels. Human exposure to aromatic hydrocarbons is regulated because of cancer risks these hydrocarbons may cause (Stetzenbach, 1986; Wilson, B. H., 1986a; Wilson, J. T. et al., 1988). Many regulatory agencies identify contamination problems and monitor the progress of treatment by measuring concentrations of only three kinds of aromatic hydrocarbons commonly found in refined fuels: benzene, toluene and xylene (BTX) (Galaska, 1989).

With water transport so important in the spread of risks to human health, and with government regulatory authority and interest concentrated on this contaminant



transport media, the historic focus of research attention and cleanup activity on water is easily explained.

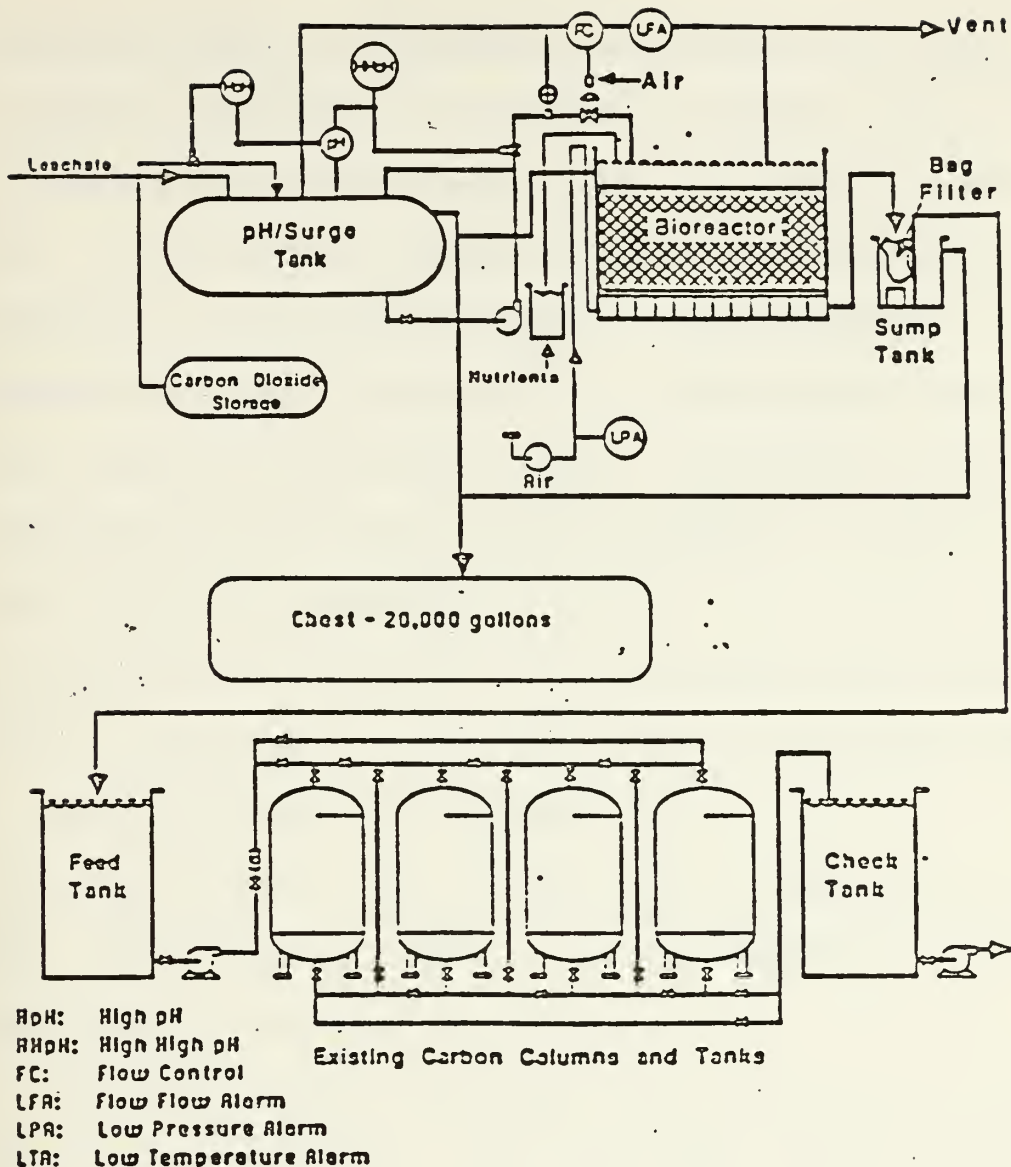
When biological methods are used for cleanup of petroleum-contaminated ground water, they are often applications of established water treatment technology (Lee and Ward, 1985). Various methods of pump-out and treatment of ground water discovered in recent literature are briefly explained below:

#### V-B-1-a-(1). Fixed-film bioreactors

Fixed-film bioreactors are devices which provide a surface to which hydrocarbonoclastic bacteria colonies attach. Mounting these surfaces inside a container, which may be a vessel as small as a 9 foot long cylinder, 6 feet in diameter, limits the extent of the bacteria colony. Within this small volume, its environment can be effectively monitored and controlled to maximize vitality within its range of tolerance while the contaminant it is cleaning is delivered in efficient concentration (Skladany et al., 1987). Figure 1 is a schematic of the equipment and flow pattern in a fixed-film bioreactor.

Aerobic bacteria colonies are often established in such reactors, yielding the requirement to monitor and ensure adequate supplies of oxygen (Stover, 1989).





Note: Contaminated water enters at the point labelled "Leachate"; CO<sub>2</sub> produced by degrading bacteria is carried out with circulating air at the point labelled "Vent".

Figure 1. Schematic of the equipment and flow pattern in a fixed film bioreactor

Source: Adapted from Skladany (1988)



Inoculant bacteria to fixed-film bioreactors may come from a variety of sources. As in other systems, they may be introduced from cultures established in reactors already in use. They may be prepared consortia developed in a laboratory (a situation exhibiting the successful "use of selected bacteria" discussed by the implementing letter to this research (Carstanjan, 1989)). Cultures may also be established from sewage or waste treatment sludges, from native bacteria resident in soils local to the site of contamination - ideally those extracted from areas of natural attenuation of the spill which are already adapted to the contaminant to be treated - or from other sources (Anon., 1988b; Anon., 1988c; Burton and Kent, 1988; Galaska, 1989). Once the degrading colony is established, treatment may commence.

Contaminated water is passed over the bacteria colonies inside the bioreactors. This water may require pretreatment. Nutrient requirements for the colony such as nitrates or phosphorus are monitored and supplied where deficient. Other pretreatments may be necessary to mitigate stress to the bioreactor microbes. These may include heating or pH buffering. The rate of water flow across the colony (generally called the biofilm in fixed-





film bioreactors) is adjusted to provide sufficient contact time between the contaminated water and the biofilm for uptake of contaminants by the resident bacteria while providing sufficient shearing force by that water's movement across the face of the biofilm to strip off dead or decaying microbes (Skladany, 1987). This stripping ensures efficient contact between contaminated water and active biodegraders. Failure to remove this excess biota (biomass) can quickly yield fouling and plugging of the bioreactor, impeding or eliminating flow of water through it.

The accumulated mass of bacteria sheared off by the flow of water over the biofilm may be filtered out of the effluent water stream before it is discharged or reintroduced to the contaminated site by surface application or subsurface injection. This filtered biomass is rated as non-hazardous waste and is easily disposed (Galaska, 1989). Gases of respiration produced by the bacteria colony (largely CO<sub>2</sub>) are often passed through an activated carbon filter to ensure volatile hydrocarbons are not released to the atmosphere (Galaska, 1989).

Effluent water from fixed-film bioreactors is commonly passed through activated carbon columns as well to remove



carry-over contamination. Given an effectively operating bioreactor, such additional treatment is in practical terms a safety device. It protects effluent quality from failure of the bioreactor which may occur from a variety of shocks, starvation, or improper operation and maintenance. Activated carbon treatment may also be a locally-approved treatment technique, where biotreatment may not. Since reduction of contaminant concentration is nearly complete in an effectively operating bioreactor, an activated carbon column down stream will be little more than a conduit for clean water and will require little if any recharging of its expensive contents during treatment. Its use in this application is prudent however, and may cause the entire system (pumps, bioreactor and activated carbon columns) to meet local requirements, with the bioreactor rated as a pretreatment process for the water stream's activated carbon columns (Galaska, 1989; Skladany, 1987).

Effluent water from the system, after filtration and passage through the activated carbon column, is monitored for quality per discharge standards. These standards will vary with the operating design or operations permit of the reactor. Where return to the contaminated soil is intended, cleanup requirements will be relatively lax. Where



discharge to surface waters will occur, especially where those waters communicate with potable water supplies, these quality standards may approximate local drinking water standards (Galaska, 1989). If discharge is to the local sewerage system, quality standards for effluent water from the bioreactor system are likely to be intermediate to the two limits just discussed.

#### V-B-1-a-(2). Decay mode bioreactors

Decay mode bioreactors are set up, their hydrocarbon degrading colonies established, and their equipment maintained and operated in much the same manner as the previously described fixed-film reactors except that they are used to treat contaminated streams whose hydrocarbon concentration is less than the level required to sustain stable, vigorous bacteria colonies. They may be characterized as intermittent continuous reactors in that a full and vigorous colony is initially established on the reaction surfaces of the vessel. Once established, low-concentration contaminated water is passed over the colony as in the fixed-film bioreactors. Initial removal efficiency is typically very high: near the limits of detection. With time, the colony will starve. Removal will be incomplete until finally, as the colony nearly dies



out, effluent contaminant concentrations will exceed discharge limits. At such time, treatment must stop. A vigorous colony must be reestablished by the same processes which yielded the original colony. Once reestablished, the low-concentration contaminated water may be introduced again and the cycle of decay, reestablishment and decay may continue (Skladany and Sullivan, 1987; Galaska et al., 1989).

#### V-B-1-a-(3). Activated sludge processing

The activated sludge process is a common waste water treatment technique in use at many municipal sewage and privately owned treatment works (POTWs). Figure 2 presents a schematic of material flow in an activated sludge system. Large ponds or tanks are used to establish a colony of bacteria uniquely adapted to remove pollutants from the waters they receive. Contact between bacteria and contaminants is maintained through continuous strong agitation by mechanical stirring or circulation of accumulated sludge and contaminated water mixture. This keeps the sludge particles and bacteria suspended and moving through the mixture while replenishing supplies of dissolved oxygen used by the degrading bacteria. Residence time in the aeration tank is carefully calculated. A balance is





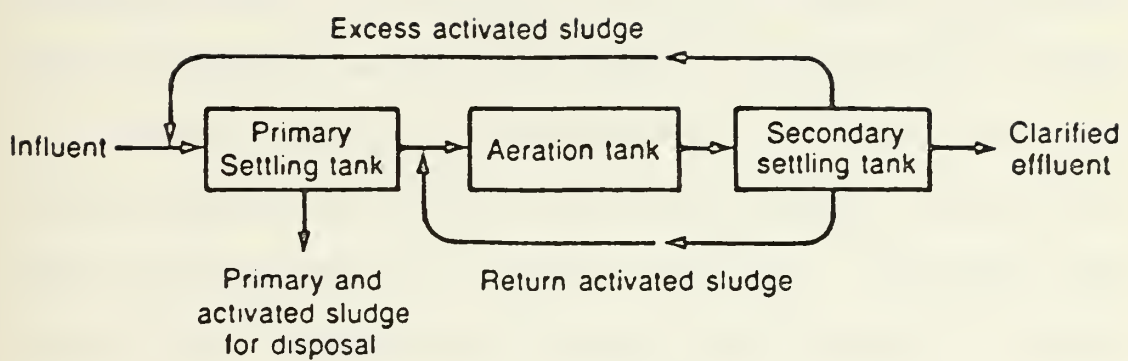


Figure 2. Schematic of the material flow in an activated sludge system

Source: Adapted from Atlas and Bartha (1987)



established between inflow and effluent once the tank is filled (Atlas and Bartha, 1987; Thomas et al., 1987a).

Pretreatment of water flowing into an activated sludge treatment tank is a common procedure at POTWs. This pretreatment often consists of a large settling tank in which heavy particulates may sink out of the relatively quiescent water. An example of another type of pretreatment is provided in the case cited by Lee and Ward in Table 1. In that situation, pretreatment by granular activated carbon ensured successful activated sludge treatment of water contaminated with organic chemicals. Effluent from an activated sludge tank is usually delivered to a second settling tank from which a portion of settled sludge, containing bacteria adapted to the contaminated stream being treated, is returned to the activated sludge aeration tank as a continuous source of reinoculation.

Just as in fixed-film bioreactors, nutrients and both physical and chemical conditions of the environment in the aeration and secondary settling tank must be carefully monitored and maintained. Although tolerant of considerable variation in flow rate and contaminant concentration, and efficient in removal of contaminants (Atlas and Bartha, 1987), activated sludge systems are sensitive to



certain inorganic contaminants, pH fluctuations, and changes in organic or hydraulic load. This is so by reason of relatively low residence time of any sample bacteria colony in the treatment tanks. The entire colony in an activated sludge system has a limited variety of degrading bacteria. Their variety is skewed toward those adapted to the steady state influent stream. Thus the range of tolerance for the colony at any instant is narrower than it would be for a colony with long exposure to altered environmental circumstances. A colony with a longer exposure time would have more opportunity to adapt and would therefore be more tolerant of change (Anon., 1987a; Anon., 1988d; Thomas et al., 1987a).

Activated sludge systems are not commonly reported as a method for removal of petroleum hydrocarbon contamination from ground water. They are however reported as a common method for treatment of petroleum refinery waste water and are applied in treatment of waste and process water from coal gasification and liquefaction (Anon., 1988d). The infrequent use of activated sludge systems for treatment of contaminated ground water is attributable to several factors. These include the relatively high capital investment the method requires, and their comparatively



high operation and maintenance costs (Anon., 1987a; Anon., 1988d). Further, sludges produced from the application of this process to water contaminated with petroleum hydrocarbons may contain refractory organic compounds posing disposal problems (Anon., 1987a; Thomas et al., 1987a). Activated sludge systems are a powerful water treatment method. Their use to treat water contaminated by petroleum wastes or spills however is cost effective only when the volume of water is very large, steady in supply, and of relatively constant contaminant concentration.

#### V-B-1-a-(4). Raw water versus waste water treatments

The treatment processes in Table 1 as reported by Maaskant are distinctly different from those in section V-B-1-a(1-3) above. The latter methods have in common their application to industrial waste water and sewage treatment situations. The treatment methods reported by Maaskant are common to potable water treatment operations. Treatments to render water potable commonly include: buffering to control pH, settling, and precipitation by flocculating agents. The use of sand filter beds is particularly common in small municipal water treatment plants. Maaskant's report of potable water processes for treatment of petroleum contaminated water was anomalous.





The use of potable water treatments is reported here for completeness of reference and as a spur to open minded assessment of treatment options.

V-B-1-b. Examples of process

Table 1, which follows, summarizes pump and treat applications of hydrocarbonoclastic bacteria to the problems of contaminated ground water.



Table 1

PETROLEUM HYDROCARBON CONTAMINATION OF GROUND WATER  
TREATED BY GROUND WATER EXTRACTION AND BIOREMEDIATION<sup>1</sup>

<u>Contaminant(s)</u> (source) (locale)	<u>Treatment</u> <u>Description</u>	<u>Ref.</u>
Gasoline (source unk) (locale unk)	Contaminated ground water pumped to submerged fixed- film bioreactors	Galaska et al. (1989) Galaska (1989)
Gasoline (tank leak) (Calif.)	Contaminated ground water pumped to a submerged fixed- film bioreactor	Skladany and Sullivan (1987)
Gasoline (tank leak) (West Va.)	Contaminated ground water pumped to a decay mode fixed-film bioreactor	Galaska et al. (1989)
Gasoline (tank leak) (Mich.)	Contaminated ground water pumped to a decay mode fixed-film bioreactor	Galaska et al. (1989)
Benzene, Toluene (source unk) (Muskegon, MI)	Activated sludge treatment preceded by granular activated carbon (GAC).	Lee and Ward (1985)
PAH * (gas works) (Netherlands)	Buffering, sedimentation, flocculation with FeCl, and sand filtration	Maaskant et al. (1986)

\* Polycyclic Aromatic Hydrocarbons

Note 1. Even where not directly reported, raw contaminants, when recoverable, are generally withdrawn from the contaminated zone by physical means, or separated from the influent stream to the treatment equipment. This action prevents overloading the degrading microbial community's capacity.



#### V-B-1-c. Evaluation of process

The pump(out) and treat(ment of ground water) process is a relatively quick but incomplete treatment method. System start-up is a matter of a few weeks versus several months for some other biotreatment methods. However, its results must be carefully interpreted (Hall, 1989). Opportunities for unscrupulous exploitation of a customer's overreaction to a contamination problem by pump and treat business operators is a clear threat. Equipment can be installed and a short-term correction of a ground water quality problem effected soon after it is discovered. For reasons discussed below, if pump and treat is not applied under very favorable conditions, contamination is likely to recur. It may then become as bad a problem as the customer faced before the expense of the pump and treat process. In application, pump and treat may meet local requirements for action in the short term. Use of this technique until complete remediation will likely cause long-term costs to be insufferably high (Mackay and Cherry, 1989).

Its use is legitimate in an integrated program of effective treatment techniques. It can control plume migration by establishing a hydraulic sink into which a contaminated aquifer may migrate. Furthermore, pump and



treat can often produce safe, clean water with less lead time than other techniques.

#### V-B-1-c-(1). Limits on method

As an exclusive treatment method, pump-out and treatment of ground water has been severely criticized. This criticism hinges on inherent limitations of this process to clean up the source of contamination. The reasons why this method does not efficiently remove the contaminant source include:

- The phenomenon of tailing: Tailing is the decline of contaminant concentration along an exponential curve towards zero as extraction continues. In practical terms, this means that ground water contaminant levels are very quickly reduced in the initial phases of pump and treat, assuming effective treatment. When treatment water is not recycled to leach out more contamination, the clean-up rate of residual petroleum contamination in contact with mobile ground water is severely limited. Inhibitors of this cleanup rate include low solubility of petroleum products in water, low mobility of petroleum in many types of soil, and practical limits on the size, configuration and flow rates in treatment reactors. The total limiting rate of cleanup is a direct function of the





maximum rate of contaminant release to ground water. This site specific rate is very low. Even with recycling of treated water, complete cleanup of the source of petroleum contaminants in an aquifer may require decades to centuries of pump-out and treatment (Hall, 1987; Hall, 1989; Hurlburt, 1987).

- Fluctuations of water table levels: soil geohydrology is very complex and will not be addressed in detail here. In general however, the water table from which ground water is extracted for treatment must remain in continuous contact with the contaminant for treatment to proceed. If it does not, treatment will be intermittent. Water table levels commonly rise and fall, often seasonally. It will usually be impractical to compensate for natural changes in the local water table through water injection or surface irrigation (Hutchins et al., 1989).

Unless soil is very porous, organic contaminants migrate through the spaces around the grains of material which make up that soil at distinctly slower rates than ground water. This diminishment, or retardation, of liquid transport rates of contaminant versus the transport rate of water (called retardation) has been observed from 0 to almost 97%. The degree of retardation depends on the soil



and liquid combination. Because of retardation, if water is pumped from the ground very quickly, the water table may drop out of contact with the contaminating petroleum. Without contact between ground water and the remaining petroleum in the soil, no petroleum hydrocarbons will pass into solution in the ground water. Contaminant removal and treatment will stop (Mackay and Cherry, 1989).

- Pooling or fugitive concentrations of contaminant: subsurface conditions may trap petroleum contaminants out of contact with mobile ground water or introduced flushing water. This is especially true in fractured rock aquifers which have had lengthy exposure to organic contaminants. In these aquifers, contaminants are able to enter dead end passageways by diffusion and remain held there by adsorption.

If the contaminated zone has clayey soil, water will pass through it slowly. Petroleum contaminants will be carried out at very low rates. These soils not only resist penetration by water but are able to strongly sorb organic contaminants (Mackay and Cherry, 1989; Stetzenbach, 1986).

V-B-1-c-(2). Prerequisites for success

Three general requirements must be met by the components of a contaminated site before the pump and treat



method can be expected to clean up the major portion of spills in the soil.

- First, the soil in the entire volume of an underground contaminant plume must be uniformly permeable.

- Second, the contaminant plume must be soluble in water or easily transported by it.

- Third, large volumes of water must be available for sacrifice to the process of contamination and cleanup.

If any one of these requirements is not met, pump and treat should not be the only remediation method (Hall, 1987).

Successful use of this treatment method to the limits of its utility occurs in response to systematic and thorough analysis of the contaminated site to determine its specific synergy of soil permeability, contaminate solubility, and water resource limitations.

V-B-2. Removal of contaminated medium and treatment at a distance from the contaminated site

As noted in Chapter II, Section B-5, those instances of petroleum contamination which have not reached surface waters or an aquifer have generally received little attention in that they were believed to pose little or no risk to human health. In very recent work, however, more



attention has been paid to contamination outside of (and most particularly above) aquifers. Such contamination reservoirs are cleaned ineffectively by pump-out and treatment of water in contact with them, and are time bombs which can produce water contamination in the future (Hall, 1987; Hall, 1989; Hurlburt, 1987; Mackay and Cherry, 1989). Treating the problem of these materials directly is more effective in the long-term than treatment of its symptoms - the most common of which is contaminated ground water (Trickett, 1989).

#### V-B-2-a. Description of process

The removal and treatment method eliminates petroleum wastes and sludges or cleans up soils contaminated with petroleum by taking these materials from their long-term storage facilities or the immediate site of contamination to a location where a microbe colony is maintained for bioremediation. This location may be a small bioreactor enclosure or a large treatment area of many hundreds of square feet. In the latter case, the method is commonly called land farming. Petroleum sludges and contaminated soils consist of various relative concentrations of petroleum, water, and solid particles. This section examines treatments for these mixtures together, since





these methods are common to wide ranges of component proportion.

During or shortly after delivering the contaminated material to the reactor vessel or land farm, nutrients and a seed culture of biodegrading bacteria are thoroughly mixed into the contaminated material. As in the case of pump and treat bioreactors, this seed culture may come from a variety of sources. In many removal and treatment events reported in the literature, continuing efforts were made to ensure uniform distribution of nutrients, contaminant, and biodegrading bacteria through the entire volume of the bioreactor. Treatments by this method are commonly done on batches of contaminated material. The treatment process may be completed in one or more stages. A major portion of contaminants may be removed in an intensive treatment reactor, with polishing to remove residual contamination at a long-term treatment site - typically a land farm

V-B-2-a-(1). Liquid/Solids Tank bioreactor (LST)

The first treatment process described in Table 2 appears to hold great promise for the Navy's backlog problem of heavy sludges or tank bottom impoundment cleanup. Figure 3 presents a flow diagram of the LST treatment method. This process is effectively a soil



Stream No.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Material									
Water (lbs.)	$22.7 \times 10^6$	$62.7 \times 10^6$	$82.2 \times 10^6$	NA	$95.8 \times 10^6$	$3.2 \times 10^6$	$25.8 \times 10^6$	$95.8 \times 10^6$	$43.0 \times 10^6$
Oil & Grease (lbs.)	$4.5 \times 10^6$	-0	$1.3 \times 10^6$	NA	-0	-0	-0	-0	-0
Solids (lbs.)	$10.0 \times 10^6$	-0	$13.2 \times 10^6$	NA	-0	-0	-0	-0	-0
Volume	18615 cy	$7.5 \times 10^6$ gal	48400 cy	NA	$11.5 \times 10^6$ gal	$0.4 \times 10^6$ gal	$3.1 \times 10^6$ gal	$11.5 \times 10^6$ gal	$5.2 \times 10^6$ gal

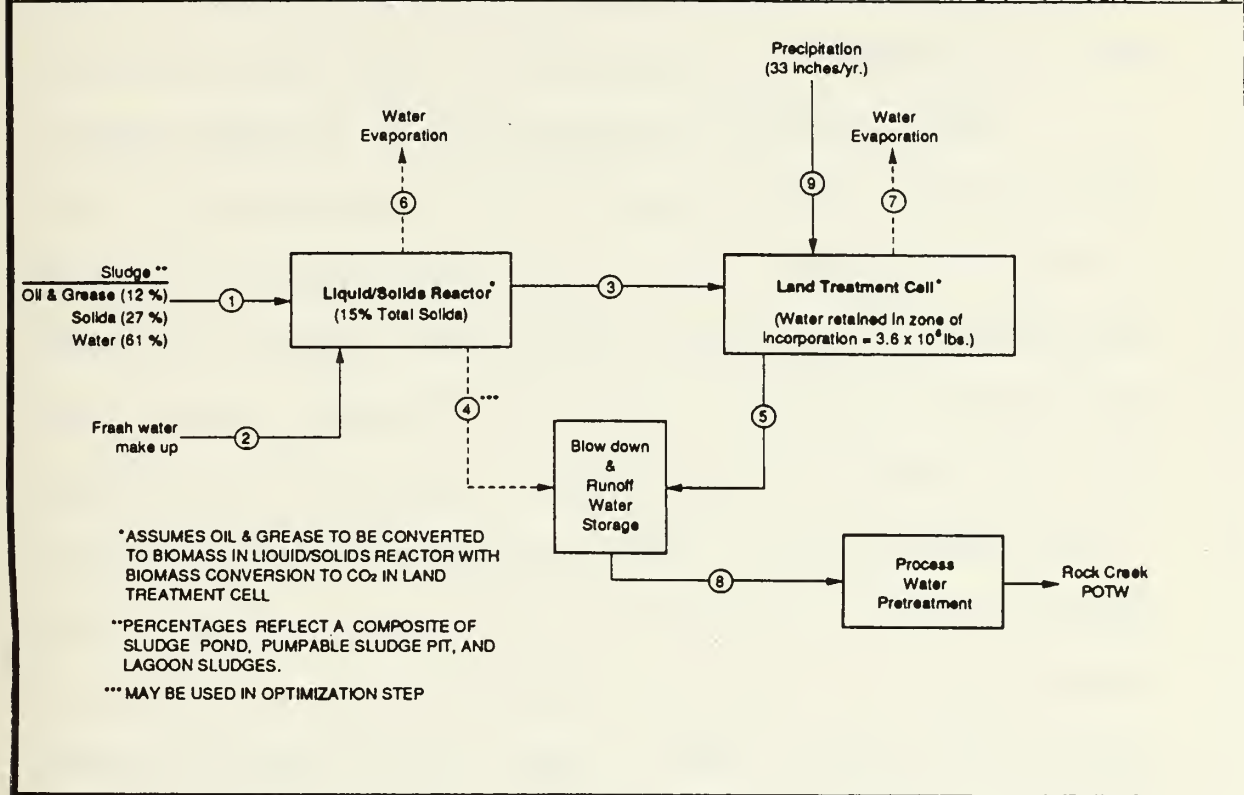


Figure 3. Flow diagram/schematic of Liquid/Solids Tank Bioreactor

Source: Adapted from Anon (1989c)



treatment system which capitalizes on the nearly complete binding of active hydrocarbon degrading bacteria to soil particles (Keely et al., 1987; Thomas, et al., 1987b). It provides excellent opportunity to monitor and correct deficiencies in the microbe's environment. Agitation of the soil in the bioreactor ensures uniform distribution of nutrients and oxygen to stimulate bioremediation. This process might be characterized as a confined land farm. The agitation and mixing provides the equivalent reactive surface area of a 31-acre surface tilled to a depth of 6 inches. This yields higher rates of hydrocarbon destruction on batch loadings of heavily contaminated material at a fraction of the capital investment and risk of a land farm of equivalent size. Volatile emissions and contaminant migrations can be controlled by confining treatment to a covered reactor built with impermeable material (Torpy et al., 1989). After a period of treatment in the reaction container (usually one summer), effluent from the bioreactor is polished with long-term traditional land farming on a "land treatment cell".

An important aspect of this method is the precedent of approval established by the EPA and various agencies of the state of Missouri, who have endorsed it as acceptable



(effectiveness aside). This precedent increases the likelihood of its approval at other sites where its effectiveness can be demonstrated. (These treatment techniques and steps are similar to a MoTec of Mount Juliet, TN process selected for the 1987 SITE [Superfund Innovative Technology Evaluation] demonstration program [Anon., 1987b; Anon., 1988a].) The demonstration test of the bioreactor for this two-step treatment process yielded a 30% to 50% reduction of "oil and grease" contaminant concentrations in two months of treatment with reduction of PAH concentrations near 80% in materials dredged from a "Sludge Pit" and a "Sludge Pond". Detailed data about the sludge material treated in the test and results of the test process are provided in Appendix A. Treatment costs by this technique are estimated to range from \$100-\$150/yd<sup>3</sup>. Although this is about double the routine operating costs of land farms, as noted before, capital costs and risk are much lower (Torpy et al., 1989).

V-B-2-a-(2). Air stripping under an impermeable cover

The second and third situations reported by Table 2 below exhibit the combined use of decontaminating microbes in soil and a method which removes volatile contaminants from soil: air stripping. In air stripping, air is forced





through the region of the contaminated material. Volatile components of the contaminant plume evaporate and are carried away by the stripping air. As an exclusive treatment method, air stripping has a long history of criticism. It is currently restricted by regulations intended to control air pollution. Without cleanup of the air which has moved through the contaminant plume, air stripping of volatile soil contaminants does not solve the problem but simply moves it to another medium (see also section V-B-2-a-(4)).

When hydrocarbon laden air from an air stripping process is passed at low velocity through a soil chamber where hydrocarbon consuming bacteria are maintained, the hydrocarbons are removed. The once-contaminated air is again clean. This technique is best suited for treatment of soils contaminated by fuel spills where the contaminant fuel has high vapor pressure, such as Aviation Gasoline, JP-4, and Motor Gasolines. Spills into the soil of heavier grades of petroleum products may be treated to a limited degree by this technique, especially if their vaporization is stimulated through heating of the contaminated soil before and during air stripping (Anon., 1987a; Carricato et al., 1988; Chowdhury, 1986).



#### V-B-2-a-(3). Simple land farming

In the experiment reported by Loehr as discussed in Table 2, reductions of hydrocarbon contamination were examined when nutrients or long-term enhancement of available oxygen levels were not provided, but wastes were simply tilled into the test plots to a depth of about 6 inches. By this low-technology process, the half-life of naphthalenes, alkanes and certain aromatics was found to be about 30 days during warm months of the year, while "oil and grease" half-lives ranged from 280 to 400 days. (Here, half-life indicates that period during which contaminant concentration decreases by 50%.) The relatively long half-lives of oil and grease emphasize the merit of an LST reactor discussed above or another effective pretreatment before contaminated material is delivered to a land farm (land treatment cell).

#### V-B-2-a-(4). Modified land farming

The remaining treatment events reported by Table 2 are variations of traditional land farming methods with certain modifications. In the Ganderkesee, FRG situation, for example, modification included controls on the two processes of cross-media contamination to which land farms are prone. These are volatilization of high vapor pressure



contaminants to the air, and leaching of contaminants through the soil of the land farm to local surface or ground water. These cross-media losses from land farms are a stimulus behind the "land ban" initiative (Field et al., 1988) as applied to petroleum hydrocarbon waste farms by the announcements of the January 1989 Federal Register (Anon., 1989b). This is especially true of volatilization losses, which the air quality sections of the EPA report to be dumping soil contamination problems into the atmosphere rather than solving them (Dunlap, 1989; Glaser, 1989; Hains, 1989; McNabb, 1989). Control techniques applied in the Ganderkesee situation reportedly capture evaporating contaminants (greenhouse cover) and intercept leachates by an impermeable liner underneath and around the treatment site. At the California site reported by Ross (Table 2), remediation levels of less than 100 parts per million were achieved during a four week evaluation test. Appropriate nutrients were monitored and added as needed. Further treatment proceeded as described in the table. The Shell Oil work summarized by Wetzel dates from the early 1970s but its conclusions have been endorsed by more recent research (Atlas, 1981; Bartha and Bossert, 1984; Sims et al., 1986). In the final two situations reported by Table



2, drilling muds were applied to unamended test plots, but all plots were rototilled three times before application and once again after application. Up to 90% remediation of hydrocarbon contamination in a one year period was observed for both Diesel and Low Toxicity Oil.

V-B-2-b. Examples of process

Table 2 below summarizes events reported in recent literature where petroleum wastes and sludges were eliminated or soils contaminated with petroleum hydrocarbons were cleaned. In all cases, these materials were moved from their storage location or taken from the immediate site of contamination. They were moved to a treatment site where bioremediating bacteria were maintained.





Table 2

PETROLEUM HYDROCARBON CONTAMINATION OF SOILS OR PETROLEUM  
SLUDGE VOLUME REMEDIATION BY EXTRACTION AND BIOTREATMENT

<u>Contaminant(s)</u> (source) (volume treated) (locale)	<u>Treatment</u> <u>Description</u>	<u>Ref.</u>
Petroleum refinery sludges and soils in contact with these sludges (Sugar Creek Refinery) (24.5 tons of oil and grease) (Independence, MO)	Liquid/Solids Tank Bioreactor - batch treatment	Anon. (1988c) Shepard (1989)
Diesel fuel (source unknown) (250 kg of soil, 10,100 ppm diesel) (Netherlands)	Soil was air-stripped in a covered basin. Stripping air was then passed through a compost filter to biologically degrade volatiles taken up from the soil	De Kreuk (1987)
Petrol (gasoline) (source unknown) (1000 tons of soil, 1,500 ppm petrol) (Netherlands)	Soil was air-stripped in a covered basin. Stripping air was then passed through a compost filter to biologically degrade volatiles taken up from the soil	De Kreuk (1987)
Petroleum Refinery Wastes (waste lagoon bottoms) (volume unk) (Ithaca, NY)	7 applications of .09% to .25% oil and grease in the zone of incorporation to 4m by 4m test plots. 1 year test period.	Loehr et al. (1986)



Table 2 continued

<u>Contaminant(s)</u> (source) (volume treated) (locale)	<u>Treatment</u> <u>Description</u>	<u>Ref.</u>
Non-chlorinated hydrocarbons (Umweitschutz Wor dGmbH) (~ 131 yd <sup>3</sup> /bed) (Ganderkesee, FRG)	On-site composting for 6 months on a liner with leachate collection and green house cover.	Nunno et al. (1988)
Petroleum Hydrocarbons (hazardous waste site) (est. 15,000 yd <sup>3</sup> (California)	Soil contaminated with 2,800 ppm petroleum hydrocarbon was dug up and spread to a depth of 30 inches over an area of approx. 4 acres in two 15-inch lifts	Ross et al. (1988)
Crude oil tank bottoms, Bunker C fuel oil (Shell Oil Co.) (volume unknown) (locale unknown)	Shell Oil Company found land-spreading application rate to be about 70 barrels per acre per month.	Wetzel et al. (1988)
Diesel Oil based drilling mud cuttings (oil wells) (volume unknown) (Newkirk, OK)	Land farming, 8 inch deep rototilling, 2% by weight application	Whitfill and Boyd (1987)
LTO*-based drilling mud cuttings (oil wells) (volume unknown) (Newkirk, OK)	Land farming, 8 inch deep rototilling, 2% by weight application	Whitfill and Boyd (1987)

\*Low Toxicity Oil (C10 - C15)



#### V-B-2-c. Evaluation of process

Removal and treatment of contaminated media has a legitimate and important place in the spectrum of bioremediation. It provides effective and direct treatment of petroleum sludges. It is an optional treatment when the volume of medium (especially soil) requiring treatment is relatively small and fully confined. Removal and treatment is more costly per unit volume treated, in both time and money, than some bioremediation methods. Extraction and transportation contribute most significantly to the additional cost of this process. Where the Navy faces a contamination problem of significant volume (100,000+ yd<sup>3</sup> of contaminated medium) and must finish its cleanup of that volume quickly (in less than one year), removal and bioremediation will not be the quick and inexpensive fix.

The benefits of this method will not be achieved without systematic and thorough evaluation of a contaminated site prior to employing it. When site evaluation reveals low soil permeability or excess contaminant concentration (as in many sludges), then bioremediation by removal and treatment may still be an option. The full benefits of biodegradation to environmentally safe materials may be gained for relatively small volumes of petroleum contam-



inated media not otherwise amenable to bioremediation. Low permeability soil can be dug up, broken apart or crushed to increase its overall permeability. Highly concentrated sludges can be diluted. Other remediation methods, however, such as those reported in section II-B-3, may emerge as more cost effective at any given site, than would be bioremediation by the removal and treatment method.

#### V-C. *In situ* Treatments

##### V-C-1. Description of process

Figure 4 provides a simplified cross-sectional views of *in situ* treatment processes. The explanatory notes of Figure 4 provide information about the various modifications to the basic treatment methods as reported in Table 3 below. Simply stated, *in situ* bioremediation achieves biodegradation of contaminants in place. It usually involves enhancing natural biodegradation by replenishment of limiting nutrients. A seed culture of microbes known to degrade the contaminant in place may be introduced if native bacteria will not work. Literature on *in situ* treatments repeatedly stresses the importance of a systematic and thorough evaluation of the contaminated site and its contaminants. This evaluation must determine whether the site is qualified for *in situ* treatment methods. Two





This schematic illustrates the use of an infiltration gallery to introduce nutrient amended water over a large surface area. In this particular case, an above ground treatment system is used on extracted water to augment the bioremediation occurring underground.

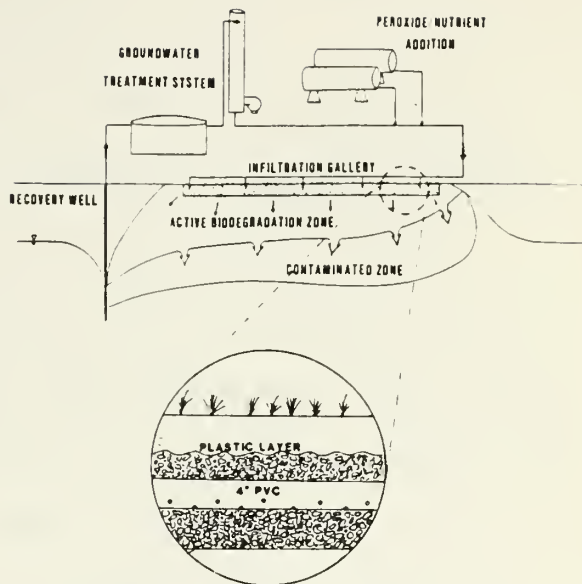
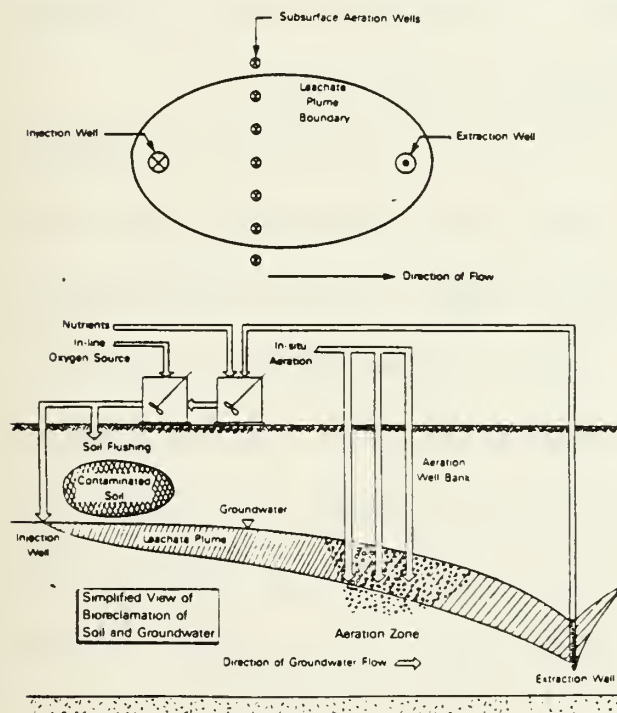


Figure 4a

Adapted from Spain et al. (1989)



A birds eye view and cross section of one combination of *in situ* bioremediation enhancement equipment is shown. In this case, nutrient amended water is introduced upgradient, while air is sparged into the contaminant plume farther downgradient. Fluid from the treatment zone is pumped out, amended with nutrients, oxygenated and reinjected.

Figure 4b

Adapted from Anon (1987a)

Figure 4.



characteristics of the site are preeminent as criteria which determine the feasibility of *in situ* bio-remediation. These characteristics are:

- The contaminated soil must be sufficiently permeable to allow entry and passage of nutrient solutions and inoculant bacteria if used (Anon., 1987a; Grubbs and Molnaa, 1988; Harris, 1987; Hilberts et al., 1985; Konieczny et al., 1985; Lee and Ward, 1985; Lee et al., 1988; Spain, 1989; Thomas et al., 1987a; Thomas and Ward, 1989; Torpy et al., 1989; Wilson, J. T. et al., 1986). As stated by Thomas and Ward, soils "with hydraulic conductivities of  $10^{-4}$  cm/sec or greater are most amenable to biore Restoration" (Thomas and Ward, 1989).

- A consortium of bacteria already adapted to or capable of adapting to the metabolization of contaminating hydrocarbons must be present (Baker et al., 1988; Chapelle and Morris, 1988; Hilberts et al., 1985; Konieczny et al., 1985; Lee and Levy, 1989; Lee and Ward, 1985; Raymond et al., 1976; Stetzenbach, 1986; Wilson, J. T. et al., 1986). If adding such bacteria is considered, these must be able to reach the contaminant and remain vigorous as they clean it up (Baker et al., 1988; Hurlburt, 1987;



Lee et al., 1988; McNabb, 1989; Wilson, J. T. et al., 1986).

#### V-C-2. Examples of process

Table 3 below summarizes examples of *in situ* treatments of contaminated soils or aquifers caused by petroleum spills on land.



Table 3

*IN SITU* BIOREMEDIATION OF CONTAMINATED SOILS AND AQUIFERS  
(Independently or in combination with other processes)

<u>Contaminant (s)</u> (source) (volume treated) (locale)	<u>Treatment</u> <u>Description</u>	<u>Ref.</u>
Gasoline (tank leak) (est. 10,000 lb) (Millville, NJ)	Product recovery followed by subsurface air sparging and nutrient injection. 6 months of treatment eliminated ground water contamination but residual gasoline was detected in effected soils.	Anon. (1985) Lee and Ward (1985) Lee et al. (1988) Raymond et al. (1978) Wetzel et al. (1987)
Petroleum Distillate (tank farm spill) (4 acre area) (locale unk)	Bioaugmentation with BI-CHEM-SUS-8 <sup>R</sup> for 21 days yielded reduction of contaminant concentration from 12,000 to less than 1 ppm	Anon. (1985)
Gasoline (source unk) (volume unk) (Granger, IN)	Product recovery followed by H <sub>2</sub> O <sub>2</sub> and nutrient amendment in line to recycled ground water	Anon. (1985)
BTX and Aliphatics (source unk) (volume unk) (Frankenthal, FRG)	Product recovery followed by nitrate addition. Ground water stripped, filtered, heated and reinjected	Anon. (1985)





Table 3 continued

<u>Contaminant(s)</u> (source) (volume treated) (locale)	<u>Treatment</u> <u>Description</u>	<u>Ref.</u>
Gasoline (pipeline leak) (est 119,000 L) (Ambler, PA)	Product recovery followed by subsurface air sparging and nutrient amendment: $(\text{NH}_4)_2\text{SO}_4$ , $\text{Na}_2\text{HPO}_4$ , $\text{NaH}_2\text{PO}_4$ . No gasoline found in extrac- ted ground water after 10 months of treatment.	Anon. (1985) Britton (1989) Lee et al. (1988) Wetzel et al. (1987)
Gasoline (tank leak) (volume unk) (La Grange, OR)	Physical recovery followed by enhancement of native hydrocarbono- clasts with oxygen by air diffusion in an injection trench, and nutrient addition to recycled ground water. No detectable hydro- carbons after 1.5 years of treatment.	Anon. (1985) Britton (1989) Lee and Ward (1985) Lee et al. (1988) Wetzel et al. (1987)
Petroleum products/ Hydrocarbons (rail yard spills) (volume unk) (Karlsruhe, FRG)	Ozonation and re- injection of ground water to enhance numbers and activity of native degrading bacteria. Ozone added at a rate of 1 g/gram dissolved organic carbon (DOC)	Anon. (1985) Britton (1989) Lee and Ward (1985)
Petroleum/ Chlorin- ated hydro- carbons (Evaporation pit) (60 ft diameter test area) (Kelly AFB, TX)	Nutrients and $\text{H}_2\text{O}_2$ added to recycled ground water.	Anon. (1985) Carricato et al. (1988) Chowdhury (1986) Wetzel et al. (1987)



Table 3 continued

<u>Contaminant(s)</u> (source) (volume treated) (locale)	<u>Treatment</u> <u>Description</u>	<u>Ref.</u>
BTX, Diesel (source unk) (volume unk) (locale unk)	Bioaugmentation by DETOX of Sugarland, TX repor- ted to effect significant reduction of contamination in 3 to 6 months	Anon. (1987a) Anon. (1988a)
Kerosine (source unk) (about 1.9M L) (New Jersey)	Liming, fertilizing and frequent tilling	Atlas (1981)
BTX (experimental application) (1800 L water spiked with 7.6 ppm BTX) (CFB Borden, Ont., Canada)	Natural attenuation monitored; only benzene persisted in a sandy aquifer after 270 days. Benzene was eliminated after 410 days. Oxygen depletion inhibited benzene degradation.	Barker et al. (1987) Major et al. (1988)
Gasoline (leakage) (volume unk) (California)	Passive monitoring of activity of naturally occurring bacteria	Britton (1989)
Gasoline (pipe break) (volume unk) (Barrow, AK)	Passive monitoring of activity of naturally occurring bacteria	Britton (1989)
Gasoline (source unk) (volume unk) (locale unk)	Ground water extracted, air stripped to remove volatiles and oxygenate, amended with nutrients and reinjected. Air also sparged into soil.	Britton (1989) Lee et al. (1988)



Table 3 continued

<u>Contaminant(s)</u> (source) (volume treated) (locale)	<u>Treatment</u> <u>Description</u>	<u>Ref.</u>
Gasoline (various source) (various volumes) (various locales)	Product recovery followed by combinations of nutrient amendment, air stripping for product removal and dissolved oxygen (DO) maintenance. DO sometimes supported with $H_2O_2$ . In one case, for treatment of about 38,000 L, air sparging into soil yielded reduction of contaminant concentration from 2,000-3,000 ppm in soil and 30-40 ppm in ground water to <50 ppm in soil and <1 ppm in ground water.	Anon. (1989) Britton (1989) Lee et al. (1988) Yaniga et al. (1985)
Mixed solvents and fuel (spill) (est. 1,100- 3,400 L hydrocarbon) (locale unk)	Nutrient solution slug injected, followed by injection of water continuously amended by $H_2O_2$ , with periodic batch additions of nutrients to this stream. Ground water extracted and polished with GAC.	Britton (1989) Lee et al. (1988)
Unleaded gasoline (spill) (6,100 kg +or- 2,500 [SD]) (locale unk)	Product recovery followed by batch addition of nutrient solution in turn followed by addition of $H_2O_2$ solution gradually increasing concentration from 0-500 ppm $H_2O_2$ .	Britton (1989) Lee et al. (1988)



Table 3 continued

<u>Contaminant(s)</u> (source) (volume treated) (locale)	<u>Treatment</u> <u>Description</u>	<u>Ref.</u>
Gasoline (source unk) (30 m thick soil layer) (locale unk)	Deep unsaturated soils vented to deliver oxygen	Britton (1989) Lee et al. (1988)
Gasoline (tank leak) (est. 45,000 yd <sup>3</sup> ) (Southern CA)	Product recovery followed by amendment of extracted ground- water with batch addition of microbial nutrients (Restore77 <sup>R</sup> ), continuous ground water perfusion to 500 ppm H <sub>2</sub> O <sub>2</sub> and rein- jection into soil upgradient.	Brubaker and Exner (1988)
Aviation Gasoline (spill) (volume unk) (U. S. Coast Guard Air Station, Traverse City, MI)	H <sub>2</sub> O <sub>2</sub> perfused water infiltrated into contaminated soil	Carricato et al. (1988)
JP-4 (spill) (unknown) (USCG AS Traverse City, MI)	Nitrate amended water infiltrated into contaminated soil	Carricato et al. (1988) Hutchins et al. (1989)
Petroleum (abandoned refinery) (volume unk) (Southern CA)	Bioaugmentation	Grubbs and Molnaa (1988)





Table 3 continued

<u>Contaminant(s)</u> (source) (volume treated) (locale)	<u>Treatment</u> <u>Description</u>	<u>Ref.</u>
Diesel (source unk) (2,000 yd <sup>3</sup> soil) (Sacramento, CA)	Bioaugmentation reduced contaminant concentration from 2,800 ppm to <38 ppm in 74 days.	Grubbs and Molnaa (1988)
Diesel (source unk) (1,500 yd <sup>3</sup> soil) (Sacramento, CA)	Bioremediation reduced contaminant concentration from 3,000 ppm to <30 ppm in about 62 days.	Grubbs and Molnaa (1988)
Weathered Statfjord crude oil (experimental application) (80 L oil/plot) (Beach, Southern Norway)	12 month test. Single 20 L/m <sup>2</sup> application of of oil and seawater emulsion in 1:1 ratio with commercial grade fertilizer on on one 4 X 2 meter test plot, and with oil soluble fertilizer on the other.	Halmo (1985)
Gasoline (pipeline spill (volume unk) (beneath an elementary school)	Physical recovery and pumping to maintain water table below school. 6 month circulation of oxygen- and nutrient-amended water eliminated detectable fuel.	Lee et al. (1988)
Scotian Shelf Condensate and Hibernia crude (experimental application) (200 ml/bag) (Eastern Nova Scotia beach, Canada)	200 ml of each type of oil added to 7 L of wet sand; mixture enclosed in mesh bags; enclosures buried flush with surface of intertidal beach sand.	Lee and Levy (1989)



Table 3 continued

<u>Contaminant (s)</u> (source) (volume treated) (locale)	<u>Treatment</u> <u>Description</u>	<u>Ref.</u>
Crude oil (source unk) (volume unk) (Supra-littoral beaches, Norway)	Oil soluble fertil- izer applied to weathered crude emulsions. 30% reduction in n-alkanes observed in one summer	Ladousse et al. (1987)
Gasoline (spill) (1,961 yd <sup>3</sup> ) (Netherlands)	1.5 year program to infiltrate water amended with by nutrients and H <sub>2</sub> O <sub>2</sub> into the contaminated zone.	Nunno et al. (1988)
Diesel Oil, Gas Oil and Crude Oil (experimental applications, spills of opportunity) (various volumes) (Spitsbergen, Norway)	Test of the effect on biodegradation rates of petroleum products intro- duced or spilled onto Arctic beaches from adding a commercial oil soluble fertilizer: INIPOL EAP22 <sup>R</sup> . to oiled Sand. 90% enhance- ment of degradation rate over 100 days reported on marine gas oil contaminated beaches.	Sveum and Ladousse (1989)



### V-C-3. Evaluation of Process

*In situ* treatment methods are promising and popular. They are most successful when applied after careful site characterization has given reliable evidence of its feasibility - an ideal *in situ* bioremediation site has permeable soil and plenty of naturally occurring aerobic hydrocarbonoclastic bacteria which require no additional nutrients. When conditions support its application, it is effective and relatively inexpensive. It is commonly a part of an integrated treatment program. This program may also include pump and treatment of ground water or removal and treatment of contaminated medium. Free product recovery frequently precedes and overlaps with the early phases of *in situ* applications.

Successful *in situ* treatments are not stable operations which can be switched on and left alone until cleanup is complete. They require careful monitoring and maintenance. When chosen, they should be expected to be a process of many months duration, proceeding at a site specific remediation rate.

*In situ* treatment methods are the subject of much contemporary research to improve their effectiveness. Several patents have been issued for variations of this



technique as a result of previous research and field applications (Dyadechko et al., 1987; Ely and Heffner, 1987; Raymond, 1974). The case summaries of Table 3 show many approaches to *in situ* bioremediation. Techniques are mixed and matched. Dogmatic adherence to one technique or other will doom its use to failure where conditions are not favorable for it (Offutt et al., 1988; Ruddiger, 1987; Stover, 1989).





## VI. COMMON ELEMENTS OF SUCCESSFUL TREATMENTS

### VI-A. Elements common to all bioremediation methods

Evident in responsible and well-engineered bioremediation projects is careful adherence to a process of project development, implementation, and application. Additionally, successful projects exhibit flexibility of their operators to respond to difficulties or failures of the design process and compensate for them. Such problems result from limitations of design data, complexity of soil geohydrology, and anomalies between laboratory and field conditions.

### VI-B. Common elements to bioremediation methods for petroleum contaminants/wastes

Of the entire series of events which comprise a bioremediation effort at a site of petroleum contamination the following elements are commonly reported:

- Product Recovery
- Site characterization
- Monitoring, maintenance and modification

#### VI-B-1. Product recovery

Efforts to reduce or limit the amount of petroleum which must be degraded by the microbe community are



routinely applied. Where raw fuel recovery is possible, whether it is mandated by regulation or not, its removal will minimize the scope and cost of a bioremediation system. The process of free product recovery gives opportunity to control where contaminant liquids are or may go. Where it will work, it is a legitimate emergency response. It is often the first remediation process, and usually continues during design and implementation of the rest of a treatment program.

#### VI-B-2. Site characterization

Site characterization is a universal element of responsibly engineered remediation projects. Included in this effort are measures to define the nature of the contaminant, the shape and volume of the contaminant plume, the speed and direction of its movement, and changes in these features (Konieczny et al., 1985; Vandegrift and Kampbell, 1988). Understanding what the plume is, where it is (the contaminated zone), and the nature of its flux is especially important in establishing how difficult to clean up it might be.

Information about the contaminated zone will probably be incomplete. It will likely be extrapolated from limited data gathered through observation at the site of contam-



ination or through laboratory studies. These extrapolations are essentially forecasts. The process of developing these forecasts will require careful and extensive sampling. These samples should consist of soil cores from the contaminated zone and its environs. These cores must be very carefully collected, transported, and stored to ensure they remain as representative of the contaminated zone as possible. Correct sampling techniques must be matched by prompt, careful analysis of the samples. Careless sampling and irresponsible laboratory work will degrade the quality of forecasts developed from their results (Dunlap, 1989; Thomas et al, 1987a; Thomas et al., 1987b; Thomas and Ward, 1989; Stetzenbach, 1986). Three important elements of site characterization will be examined in the next few paragraphs. They are:

- soil geohydrology characterization
- microbial characterization
- nutrient characterization.

#### VI-B-2-a. Soil geohydrology characterization

The interaction of soil and liquids is enormously complex. Soil geohydrology characterizations are studies of these interactions. Emerging from these studies are forecasts of what has and might yet happen to liquids in a



particular soil. For all treatment methods, the reliability of these forecasts decreases dramatically with increasing plume volume (Keely et al., 1986). Although computer modeling and field simulations of conditions in the contaminated zone are helpful, at this writing, neither of these tools are as reliable as studies of samples from the contaminated site (Keely et al., 1986; Rifai et al., 1988; Sveum and Ladousse, 1989).

Soil geohydrology characterizations give the strongest evidence of the feasibility of *in situ* treatments, although promising soil geohydrology forecasts will not guarantee success for *in situ* bioremediation methods (Anon., 1985; Konieczny et al., 1985; Ruddiger, 1987). By default, these studies may indicate the need for Removal and Treatment if bioremediation is intended. These studies can also show if the pump and treat method will have remedial effect.

#### VI-B-2-b. Microbial characterization

Microbial characterization identifies whether bacteria which already can or may be able to destroy the contaminant(s) are present in a contaminated zone. A common method to this end is the laboratory culture of small samples of contaminated soil (Leach et al., 1988; Thomas and Ward,





1989). Once developed, these small cultures (microcosms) are incubated to closely approximate *in situ* conditions. Bacteria which grow in these microcosms are presumed to be representative of the *in situ* community (Keely et al., 1986; Thomas et al., 1987b). If the microbes identified are not known to be hydrocarbonoclastic, their potential to achieve this capability or assist other known oil degrading bacteria may be evaluated. Where they are determined to be hydrocarbonoclastic, the process of nutrient characterization can begin.

Since microbial characterization produces important information about the rate and effectiveness of natural and enhanced bioremediation, it is a crucial part of *in situ* biotreatments. As discussed in Section V-C-1, stimulation and control of naturally occurring bacteria is what *in situ* bioremediation is all about (Leach et al., 1988; Thomas and Ward, 1989). *In situ* bioremediation by enhancement of natural microbes may be avoided in those cases where microbial characterization indicates natural bacteria are absent or ineffective. On the other hand, these studies may reveal that naturally occurring bacteria will solve the problem without enhancement. In such circumstances, costs would be limited to those of monitoring and follow-up. For



developers of pump and treat systems or removal and treatment facilities, these studies may reveal whether the contaminated zone can provide inoculant bacteria.

#### VI-B-2-c. Nutrient characterization

The goal of bioremediation is to bring the right microbes into contact with contaminants, while ensuring their use of it as a substrate is limited only by the contaminant's availability. To do this, the limiting nutrients other than the hydrocarbons must be known and their limiting concentrations determined. Nutrient characterization provides this knowledge. Biodegrading bacteria are reported to require nutrients of two types:

- Electron receptors
- Inorganic nutrients

Selected information about these two types of nutrients follows.

##### VI-B-2-c-(1). Electron receptors

As discussed in the early portions of this document (section II-D-2), most bacteria intended for use in bioremediation of petroleum contaminants need an electron receptor to metabolize hydrocarbons (Swindoll, 1988; Thomas and Ward, 1989). Historically, the most common electron receptor provided in one form or other is oxygen. Vigorous



aeration is an important component of the various techniques of the Removal and treatment category (section V-B). Delivery of oxygen as an electron receptor is an especially important part of *in situ* treatment methods. It is expressly cited as the electron receptor in 16 of the cases reported by Table 3. Most early and some current *in situ* bioremediations of petroleum contaminants apply the first Raymond patented process (Raymond, 1974). This technique introduces compressed atmospheric air into the contaminated zone to augment natural oxygen supplies.

Although the atmosphere is a cheap and accessible source of oxygen, aeration is unlikely to provide optimum concentrations of this electron receptor in subsurface biodegradation environments (Lee et al., 1988). Oxygen is not the majority component of the naturally occurring atmosphere. It has relatively low solubility in water. These factors limit the rate by which air or water can deliver oxygen to biodegradation microsites. For *in situ* methods,  $H_2O_2$  has been introduced to water delivered for enhancement of bioremediation in petroleum-contaminated soils or aquifers to compensate for oxygen's low solubility. Water can retain a high concentration of  $H_2O_2$ . As bacteria use dissolved oxygen from peroxidized water, the chemistry of



$H_2O_2$ 's dissolution balance drives more oxygen into solution in the water. Bacteria can be acclimated to tolerate concentrations of  $H_2O_2$  which yield 50 times greater oxygen availability than can be achieved even by direct solution of pure oxygen. Using  $H_2O_2$  solutions ensures biodegradation processes will not be limited by electron receptors availability - natural processes will be enhanced. This relatively recent development (since early 1980s) is reported in several treatments of Table 3 (Anon., 1987a; Lee et al., 1988; Thomas and Ward, 1989).

Water with high concentrations of  $H_2O_2$  can be lethal to bacteria. The degrading colony must be carefully acclimated to elevated  $H_2O_2$  concentrations (Anon., 1985; Lee et al., 1988; Thomas and Ward, 1989). Very high concentrations of  $H_2O_2$  in water may also cause precipitation or mobilization of minerals in the soil. This may in turn reduce soil permeability or foul treatment equipment. These side effects may cause  $H_2O_2$  solutions to be an unacceptable method of enhancing the natural levels of electron receptors in a contaminated zone.

Several recently published reports and some current research projects focus on delivery of other electron receptors which have high solubility in water, but do not





produce the unwanted side effects of  $H_2O_2$  solutions (Kuhn et al., 1988; Major et al., 1988; Thomas and Ward, 1989; Wilson, B. H. et al., 1986a; Wilson, B. H., 1986b; Zeyer et al., 1986). Nitrate compounds are commonly used to this end. Nitrate amendment is not without its own risks. Nitrate concentrations greater than 10 mg/l violate the Federal Drinking Water Standards (Anon., 1976; Anon., 1985). Escape of nitrate-amended water from the treatment zone could contaminate local aquifers (Hutchins, 1989; Lee and Ward, 1985; Mayfield, 1989).

#### VI-B-2-c-(2). Inorganic nutrients

Even if water with elevated electron receptor concentrations can be delivered to the potentially degrading bacteria, other requirements of bioprocess for optimal hydrocarbonoclastic activity may be lacking where required at the degrading site. Laboratory studies with cultures of native or adapted bacteria interacting with the contaminant to be removed can determine optimal combinations of nutrients. Most commonly, these studies reveal a need for sources of inorganic nitrogen and phosphorus (Anon., 1985; Anon., 1987a).

Inorganic nutrients are commonly provided in pump and treat systems by mixing them into the contaminated water



upstream of the bioreactor. For relocation and treatment methods, these nutrients may be provided as a solution which is mixed into the bioreactor volume or sprayed onto the surface of a land farm. They may also be delivered by commercial fertilizers sprinkled on or mixed with the volume of material in treatment.

For *in situ* methods, as seen from the several examples of nutrient amendment in Table 3, delivery of other nutrients into the contaminated zone is an important element of bioenhancement. For these treatments, a ratio of organic carbon to available nitrogen and phosphorus of 300:15:1 has been reported as minimal (Konieczny, 1985). This may be compared with a carbon to nitrogen ratio reported as recommended for land farming of 160:1 and laboratory experiments which reported carbon to nitrogen ratios of 60:1 and carbon to phosphorus of 800:1 to be optimal (Grubbs and Molnaa, 1988). *In situ* nutrients amendments are commonly delivered in batch quantities. A well-dispersed batch amendment will be provided before introducing a continuous source of electron receptors (oxygen). This avoids a bloom of microbes which might plug the soil and impede further flow of oxygen and nutrients (Thomas and Ward, 1989).



The optimal concentrations and delivery rates of the necessary nutrients are site-specific. They are probably microsite-specific within the treatment zone. Once the nutrient requirements of the biodegrading bacteria colony are determined, a trade-off will need to be developed between optimally meeting its needs and the delivery capabilities of the treatment system and its operators.

#### VI-B-3. Monitoring, maintenance and modification

Once applied, monitoring, maintenance, and modification of process in response to situation changes or new information are essential to successful bioremediation.

Bioremediation can achieve reduction of contaminant concentrations. Reports of cleanup to declared standards or even to levels at or below background contamination are common enough to be encouraging. Bioremediation does not proceed automatically however. Its success is limited when its application is not monitored, and weaknesses in design or process identified and corrected. Where cleanup, as measured by the rate of reduction in contaminant concentration, has levelled off, the treatment in place should be reviewed. Continued use of the existing system, a modification to it, or use of an alternate process should be carefully evaluated (Thomas and Ward, 1989).



## VII. ERRORS OF PROCESS OR TREATMENT

### VII-A. Ineffective Bioaugmentation

Where native bacteria do not exhibit the ability or capability of degrading the contaminant of interest, inoculation with bacteria known to be capable of the required degradation might be considered. Such inoculations are often referred to by the term bioaugmentation. It is unlikely that bacteria introduced into a petroleum-contaminated zone in the environment will be the sole source of decontamination action. Biodegradation of petroleum in soils is known to be the symbiotic activity of consortia of bacteria unique to each contaminated site (Lee et al., 1988). Bacteria which can degrade a weathered contaminant at each microsite in a contaminant plume will not be produced in a laboratory (bioengineered). Further, in subsurface environments, the ability of bioengineered bacteria to survive, to escape predation by protozoa, to be transported into the contaminated zone and to retain their bioengineered degrading capability is severely limited (Baker, 1988; Dunlap, 1989; Knapp, 1989; Lee et al., 1988; McNabb, 1989). These engineering factors aside, public relations problems and local regulations further limit





introduction of specialized bacteria into soils or contaminated aquifers for biotreatment (Wilson, J. T. et al., 1986). Therefore, even where it can be used, the effectiveness of bioaugmentation is severely limited.

Success with bioaugmentation is in direct proportion to the degree to which the introduced microbe colony can be protected from the natural environment. Bioaugmentation is most successful when used on confined sites of waste accumulation such as tank bottoms or ships' bilges (Dunlap, 1989; Knapp, 1989; McNabb, 1989).

#### VII-B. Incomplete Soil Geohydrology Characterization

In one of the cases reported by Table 3 (Britton, 1989; Lee et al., 1988) the natural permeability of the soil was very low. Nevertheless, *in situ* treatment by point injection of  $H_2O_2$  solutions was applied. Since this solution could not quickly flow away from the injection points, pools of nutrients developed around them. Blooms of bacteria resulted around these pools. Their biomass plugged fluid transport pores in the soil near these points, impeding delivery of nutrient amendments. Clean-up in areas away from injection points was not enhanced. Introduction of a high-concentration  $H_2O_2$  solution cleared this plugging and restored some enhancement at a distance



from the injection points. In those distant zones,  $H_2O_2$  concentrations, now lowered by reason of oxygen consumption in cleaning out the fouling microbes, were within the tolerance range of actively degrading bacteria. However, the remediation rate was still slow. Soil geohydrology characterization was incomplete in this case. Evidence of low soil permeability was not accommodated in the treatment system design. Not only must data from characterizations be developed; it must be prudently used.

#### VII-C. Failure to Monitor/Modify Applied Methods

In one case cited in Table 3, a 63% reduction in unleaded gasoline concentrations was achieved by enhanced biodegradation during the intended treatment period but residual concentrations in extracted ground water measured from 1 to 3 parts per thousand. With target cleanup concentrations for ground water measured in hundreds of parts per billion (Wilson, B. H., 1986a; Wilson, J. T. et al., 1988), evaluation of further treatment by this method or by an alternate technique was required (Lee et al., 1988).

If peroxidation of injected or infiltrated water is an element of the treatment process, *in situ* concentrations must be carefully monitored. High concentration will cause



escape of  $H_2O_2$  by dissolution. It may also cause precipitation of minerals or other *in situ* chemical reactions. The formation of precipitates may cause plugging of soil pores, reducing soil permeability. Ideally, the  $H_2O_2$  concentration will balance the biologic oxygen demands of the degrading community with no other effect (Anon., 1985; Anon.; 1987a, Spain et al., 1989).

Examples of the problems which can occur and must be solved for effective bioremediation, when delivery of peroxidized water *in situ* is part of a treatment system, are evident in the Kelly AFB effort reported by Table 3. Precipitation resulting from chemical reactions between  $H_2O_2$  solutions and *in situ* soil minerals was very severe. Precipitates virtually plugged the treatment zone, reducing infiltration capacity by 90%. Further, metal sediments were found inside surface equipments, after treatment had proceeded for a time, transported there with water extracted from the treatment zone for nutrient amendment, peroxidation and reinjection. Such metal sediments may themselves be hazardous wastes requiring special handling and disposal (Wetzel et al., 1987).



In all cases, careful attention must be given to detecting and correcting problems with bioremediation treatment systems as occurring.

VII-D. Failure to Coordinate with the Robert S. Kerr  
Environmental Research Laboratory.

It is likely that some time will pass before sufficient expertise is developed within the Navy to engineer bioremediation systems. This expertise must include the ability to evaluate the applicability of bioremediation to Navy petroleum wastes or petroleum-contaminated sites, and design bioremediation systems. After implementation, the bioremediation engineer should monitor and modify that system as necessary to achieve successful cleanup. During the period in which this expertise develops, the Navy should avoid the problems encountered in previous bioremediation projects of the Department of Defense and avoid duplicating work already complete (Carricato et al., 1988; Dunlap, 1989; McNabb, 1989). As reported by their mission statement, the Robert S. Kerr laboratory is

EPA's center for ground water research, focusing its efforts on...development of methodologies for protection and restoration of ground water quality, and evaluation of the applicability and limitations of using natural soil and subsurface process for the treatment of hazardous wastes...responsibilities have included the development and demonstration of cost effective methods for (treatment) of petroleum refining and petrochemical wastes.





They further report their activities as of 1989 include

Development of remediation technologies which are effective in protection and restoring ground water quality without being unnecessarily complex or costly, and without unduly restricting other land use activities.

They are the Federal activity responsible and funded for development of remediation processes and systems. They are the legitimate source of assistance to the Navy's efforts in this area.



## VIII. CONCLUSIONS

1. Bioremediation is not universally applicable to petroleum-contaminated sites or to the treatment of petroleum wastes. Biotreatment of petroleum spills or sludges can be a tool of great power and utility, but successful application follows systematic and careful evaluation of all constraints. These include not only limits on engineering and biotechnology, but also those political and temporal.
2. At any given contaminated site, all optional treatment techniques should be evaluated against engineering and political limits. Most critical among the engineering limits for bioremediation will be constraints of geohydrology and time. Political and legal constraints may preclude bioremediation entirely.
3. The geohydrology of petroleum contaminated soil is the preeminent engineering factor which determines the feasibility of bioremediation. If liquids cannot be transported to, through, and removed from the contaminated medium/zone, then



petroleum contamination will resist extraction for pump and treatment and natural rates of *in situ* bioremediation of contaminants will resist enhancement.

4. The interrelationship of soil geohydrology, microbial degradation of contaminants, and the need for nutrients to accomplish that degradation is very complex. Given permeable soil, and a bacteria colony in it adapted to or adaptable to degrading petroleum contaminants, enhancement of their activity may be possible. Water containing nutrients which enhance biological activity can increase petroleum biodegradation rates if it can reach the contaminant plume.
5. Certain chemicals become more toxic when subject to biodegradation. The concentration of such chemicals in material to be cleaned up by bioremediation must be evaluated. The impact of harmful biodegradation products must be determined. If it produces environmentally significant quantities of more toxic contaminants, the application of bioremediation will not be useful.
6. The merit of bioaugmentation treatments *in situ*



is debatable. In that environment, the effectiveness of bioaugmentation is severely limited by constraints of biology, biotechnology and politics.





## IX. RECOMMENDATIONS

- I recommend the U. S. Navy avoid applying bioremediation as a universal solution to environmental problems of petroleum contamination. At any given site, all optional treatment techniques should be evaluated against engineering and political limits.

- I recommend a treatment guide be developed for use at Navy fuel terminals. Its use would assure systematic and thorough evaluation of contaminated sites or materials to determine which remediation method meets local needs.

- As a project or thesis of a Navy Petroleum Management student at the University of Kansas, I recommend preparation of a bioremediation guide. Its use would allow evaluation of bioremediation as a solution to a petroleum waste or contamination problem. This guide would eventually be part of the total treatment guide.

- Until bioremediation expertise is developed within the Navy, I recommend that early development of technique and processes in this field be coordinated with the United States Environmental Protection Agency's Robert S. Kerr Environmental Research Laboratory in Ada, Oklahoma.



## REFERENCES

Anonymous (1976); National Interim Primary Drinking Water Regulations (U.S.E.P.A. 1976) cited in Kansas Surface Water Quality Standards; State of Kansas, Department of Health and Environment; May 1, 1987, Section III, Table 4; 28-16-18e; p. 7

Anonymous (1985); Handbook; Remedial Action at Waste Disposal Sites (Revised); U. S. Environmental Protection Agency, Office of Emergency and Remedial Response Washington D.C./Office of Research and Development, Hazardous Waste Engineering Research Laboratory; Cincinnati, OH; EPA/625/6-85/006; October 1985

Anonymous (1987a); Handbook Groundwater; U. S. Environmental Protection Agency, Office of Research and Development, Center for Environmental Research Information, Cincinnati, OH/Robert S. Kerr Environmental Research Laboratory; EPA/626/6-87/016

Anonymous (1987b); "Superfund Innovative Technology Evaluation (SITE) Program report (EPAX88030012)"; presented at HMCRI Conference November 16-18, 1987; U. S. Environmental Protection Agency; pp. 7-9

Anonymous (1988a); "BIODEGRADATION"; Hearing before the Subcommittee on Energy and Agriculture of the Agriculture of the Committee on Small Business; House of Representatives, One Hundredth Congress, Serial No. 100-27, Hearing held September 30, 1987; U. S. Government Printing Office

Anonymous (1988b); "Design, operating and closure plan for a biological treatment demonstration project"; prepared for AMOCO Oil Company Chicago, IL by Remediation Technologies; Austin, TX

Anonymous (1988c); "Final report, biological treatment demonstration project, biological treatment of petroleum refinery sludges from the Sugar Creek facility, tank scale study"; prepared for AMOCO Oil Company Chicago, IL by Remediation Technologies; Austin, TX



- Anonymous (1988d); Energy Technology in the Environment; Department of Energy; Report No. DOE-EH-0077 prepared for the Department of Energy by Argonne National Laboratory; Washington, DC; Chapter 11, pp. 171-214
- Anonymous (1989a); "California Approves Closure of Bioremediation Project"; JAPCA, The Journal of the Air & Waste Management Association, Vol 39, No 5, May, 1989; Air & Waste Management Association; p. 572
- Anonymous (1989b); Federal Register; January 11, 1989; Part 3, pp. 1055-1120
- Anonymous (1989c); Operating Plan for the Closure of the Sludge Pond, Sludge Pit and Wastewater Treatment Lagoon as a Single Waste Management Unit; report submitted to Missouri Department of Natural Resources and U. S. Environmental Protection Agency, Region VII, Vol. 1, AMOCO Oil Company, Chicago, IL.
- Atlas, R. M. (1981); "Microbial Degradation of Petroleum Hydrocarbons: an Environmental Perspective"; Microbiological Reviews, Vol. 45, No. 1, March, 1981; pp. 180-209
- Atlas, R. M. editor (1984); Petroleum Microbiology; Macmillan Publishing Company; New York, NY and London, U.K.
- Atlas, R. M. in Omenn, G. S. (ed) (1988); "Biodegradation of Hydrocarbons in the Environment"; Environmental Biotechnology, Reducing Risks from Environmental Chemicals through Biotechnology, Basic Life Sciences Series, Volume 45; Plenum Press; New York, NY and London, U.K.; pp. 211-222
- Atlas, R. M. and Bartha, R. (1987); Microbial Ecology: Fundamentals and Applications (Second Edition); The Benjamin/Cummings Publishing Company, Inc.; Menlo Park, CA; p. 233
- Baker, K. H.; Herson, D. S.; Buniski, D. A. (1988); "Bioremediation of Soils Contaminated with a Mixture of Hydrocarbon Wastes: A Case Study"; Superfund '88, Proceedings of the 9th National Conference, Nov 28-30, 1988, Washington, D.C.; Hazardous Material Control Institute; pp. 490-494



- Barker, J. F.; Patrick, G. C. and Major, D. (1987); "Natural Attenuation of Aromatic Hydrocarbons in a Shallow Sand Aquifer"; Ground Water Management Review, Winter, 1987; pp. 64-71
- Bartha, R. and Bossert, I. in Atlas, R. M. editor (1984); Petroleum Microbiology; Macmillan Publishing Company; New York, NY and London, U.K.; Chapter Fourteen, pp. 570-571
- Bowlen, G. F.; Kosson, D. F. (1988); "Anaerobic Biodegradation of Benzene, Toluene and Xylenes"; Superfund '88, Proceedings of the 9th National Conference, Nov 28-30, 1988, Washington, D.C.; Hazardous Material Control Institute; pp. 451-454
- Britton, L. N. (1989); "Aerobic Denitrification as an Innovative Method for *In Situ* Biological Remediation of Contaminated Subsurface Sites (Final Report for the Period May 1986 - November 1987) ESL-TR-88-40"; Engineering & Services Laboratory, Air Force Engineering & Services Center; Tyndall Air Force Base, FL
- Brubaker, G. R. and Exner, J. H. in Omenn, G. S. (ed) (1988); "Bioremediation of Chemical Spills"; Environmental Biotechnology, Reducing Risks from Environmental Chemicals through Biotechnology, Basic Life Sciences Series, Volume 45; Plenum Press; New York, NY and London, U.K.; pp. 163-171
- Burton, R. E. and Kent, W. D. (1988); "Recycling of the aqueous fraction of organic toxic wastes and biological conversion of the solid fraction"; Proceedings of the Fourth Annual Hazardous Materials Management Conference/West, part of the Hazardous Materials Management Conference & Exhibition/West, Long Beach, CA, November 8-10, 1988; HazMAT88/West; pp. 437-447
- Carricato, M. J.; Hill, R. D.; Hindman, T. B. (1988); Third Annual Report of the Interagency Work Group on Hazardous Waste Technologies convened 21-22 June, 1988; HAZWRAP Support Contractor Office of Martin Marietta Energy Systems, Inc.; Oak Ridge, TN; December, 1988





- Carstanjan, J. H. (1989); personal correspondence as Commanding Officer, Navy Petroleum Office, Cameron Station, Alexandria, Va of 20 March, 1989
- Chapelle, F. H.; Morris, J. T. (1988); "Potential for *In situ* Biodegradation of JP-4 Jet Fuel at the Defense Fuel Supply Point, Charleston, South Carolina"; United States Department of the Interior, U. S. Geological Survey
- Chowdhury, J. (1986); "CPI go below to remove groundwater pollutants"; Chemical Engineering, V 93, No 111986, June 9, 1986; pp. 14-19
- De Kreuk, J. F. in Assink, J. W.; Van Den Brink, W. J. (editors) (1986); "Microbial Decontamination of Excavated Soil"; Contaminated Soil; Proceedings of the First International TNO Conference on Contaminated Soil, (11-15 November, 1985, Utrecht, The Netherlands) Marinus Nijhoff Publishers; Dordrecht, The Netherlands; pp. 669-678
- Dunlap, W. (1989); personal communications during interviews; Robert S. Kerr Research Laboratory, Ada OK; 10-13 July, 1989
- Dyadechko, N.; Nesterov, I. I.; Tolstokorova, L. E.; Platonova, S. V. (1987); "Bacterial Composition and Method for Purifying Water and Soil of Oil Pollution"; PCT Gazette, No. 27/1987, December 03, 1987; p. 4703
- Ely, D. L.; Heffner, D. A. (1987); "Process for *In Situ* Biodegradation of Hydrocarbon Contaminated Soil"; U. S. Patent 4,765,902 C8/23/88 F 9/25/87
- Field, S. D.; Forbes, L.; Marks, R. E.; Wojtanowicz, A. K. (1988); "Biological Treatment of Oilfield and Petrochemical Hazardous Wastes"; Proceedings of the Engineering Foundation Conference on Biotechnology Applications in Hazardous Waste Treatment, October 30-November 4, 1988, Longboat Key, Florida; The Engineering Foundation
- Galaska, E. G. (1989); personal communication during interview at DETOX Inc. offices; Dayton, OH; 25 May, 1989



- Galaska, E. G.; Skladany, G. J.; Nyer, E. K. (1989); "Biological Treatment of Groundwater, Soils and Soil Vapors Contaminated with Petroleum Hydrocarbons"; Proceedings of the 44th Annual Purdue Industrial Waste Conference, Purdue University, West Lafayette, Indiana; Lewis Publishers; Chelsea, MI
- Glaser, J. A. (1988); Promising Technologies for the Biological Detoxification of Hazardous Waste, EPA/600/D-88/040; Hazardous Waste Engineering Research Laboratory, U. S. Environmental Protection Agency, Cincinnati, OH
- Glaser, J. A. (1989); personal communication during interviews at United States Environmental Protection Agency, Risk Reduction Engineering Laboratories; Cincinnati OH; May, 1989
- Glaser, J. A.; Lamar, R.; Dietrich, D.; Kirk, T. K. (1989); "Treatment of Wood Preserving Soil Contaminants by White Rot Fungus"; paper presented to the Fifteenth Annual Research Symposium on Remedial Action, Treatment and Disposal of Hazardous Waste, 10-12 April, 1989; Risk Reduction Engineering Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, OH
- Gosse, M. M.; Farrell, L. L.; Prominski, N.; Arienti, M.; Dwight, D. (1985); "Corrective Measure for Releases to Ground Water from Solid Waste Management Units, Draft Report (Final); EPA/530/SW-88/020"; prepared for U. S. Environmental Protection Agency by Alliance Technologies Corp.; Bedford, MA
- Grbic-Galic, D. and Vogel, T. M. (1987); "Transformation of Toluene and Benzene by Mixed Methanogenic Cultures"; Applied and Environmental Microbiology, Vol. 53, No 2. Feb. 1987; American Society for Microbiology; pp. 254-260



- Grubbs, R. B. and Molnaa, B. A. (1988); "In Situ and On-Site Biological Treatments of Contaminated Soils Which Utilize Microbial Consortia Inoculants"; Proceedings of the Fourth Annual Hazardous Materials Management Conference/West, part of the Hazardous Materials Management Conference & Exhibition/West, Long Beach, CA, November 8-10, 1988; HazMAT88/West; pp. 131-144
- Hains, J. (1989); personal communication during interviews at U. S. Environmental Protection Agency Research Laboratories, Cincinnati, OH; May, 1989
- Hall, C. W. (1985); Key note speech to Fifth National Symposium on Aquifer Restoration and Ground Water Monitoring; Proceedings of the Fifth National Symposium on Aquifer Restoration and Ground Water Monitoring held 21-24 May, 1985, Columbus, OH; National Well Water Association; Worthington, OH; pp. 7,11
- Hall, C. W. (1987); "Practical Limits to Pump and Treat Technology for Aquifer Remediation"; Paper prepared for the U.S. Environmental Protection Agency, at Robert S. Kerr Environmental Research Laboratory Ada, OK
- Hall, C. W. in Marbek Resource Consultants, Ltd. (1989); "U. S. EPA R&D Efforts and Activities in Groundwater and Soil Remediation"; Remediation Technologies for Treating Hydrocarbon Contaminated Groundwater and Soil: AN R, D & D PLAN OF ACTION. A report on meetings held in Collingwood, Ontario 11/28-12/1, 1988 and Calgary AL 1/11/89; Waste Water Technology and Industrial Programs Branch, Environment Canada et al.; pp. 16-17
- Halmo, G. (1985); "Enhanced Biodegradation of Oil"; Proceedings of the 1985 Oil Spill Conference, American Petroleum Institute; pp. 531-537
- Harris, M. R. in Cairney, T. (editor) (1987); "Recognition of the Problem"; Reclaiming Contaminated Land; Blackie and Son Ltd.; Glasgow and London, U.K.; Ch. 1



- Hater, G. R. (1988); "Bioremediation of *In situ* and *ex situ* contaminated soils"; Proceedings of the Fourth Annual Hazardous Materials Management Conference/West, part of the Hazardous Materials Management Conference & Exhibition/West, November 8-10, 1988 Long Beach, CA; HazMAT88/West; pp. 234-240
- Hilberts, B.; Eikelboom, D. H.; Verheul, J. A. H. M. and Heinis, F. S. in Assink, J. W.; Van Den Brink, W. J. (editors) (1986); "Microbial Decontamination of Excavated Soil"; Contaminated Soil; Proceedings of the First International TNO Conference on Contaminated Soil, (11-15 November, 1985, Utrecht, The Netherlands) Marinus Nijhoff Publishers; Dordrecht, The Netherlands; pp. 679-691
- Hurlburt, S. (1987); "Raymond Approaches Hydrocarbon Spills Head-On"; Groundwater Management Review, Spring 1987; pp. 90-93
- Hutchins, S. R. (1989); personal communications during interviews; Robert S. Kerr Research Laboratory, Ada OK; 11 and 12 July, 1989.
- Hutchins, S. R.; Wilson, J. T.; Douglass, R. H.; Hendrix, D. J. (1989); "Field and Laboratory Evaluation of the Use of Nitrate to Remove BTX from a Fuel Spill"; presented at the Environmental Research Conference for Ground Water Quality and Disposal, Washington, D.C., May 2-4, 1989
- Keely, J. F.; Piwoni, M. D.; Wilson, J. T. (1986); "Evolving concepts of subsurface contaminant transport"; Journal Water Pollution Control Federation, May, 1986; pp. 349-357
- Knapp, R. (1989); personal communications during telephone interview 18 May, 1989
- Konieczny, S. C.; Farrell, L.; Gosse, M. M.; Myatt, B.; Kay, B.; Bell, R.; Murphy, T.; Ram, N. M. (1985); "Corrective Measures for Releases to Soil from Solid Waste Management Units" Draft report (Finalized August, 1985); for the U. S. Environmental Protection Agency, Land Disposal Branch, Washington D.C. by Alliance Technologies Corporation; Bedford, MA; EPA/530/SW-88/022





- Kuhn, E. P.; Zeyer, J.; Eicher, P. and Schwarzenbach, R. P. (1988); "Anaerobic Degradation of Alkylated Benzenes in Denitrifying Laboratory Aquifer Columns"; Applied and Environmental Microbiology, Vol. 54, No. 2; pp. 490-496
- Ladousse, A.; Tallec, C.; Tramier, B. (1987); "Progress in Enhanced Oil Degradation"; Proceedings, 1987 Oil Spill Conference (Prevention, Behavior, Control, Cleanup) held April 6-9, 1987, Baltimore, MD; p. 625
- Leach, L. E.; Beck, F. P.; Wilson, J. T. and Kampbell, D. H. (1988); "Aseptic Subsurface Sampling Techniques for Hollow-Stem Auger Drilling"; Proceedings of the Second National Outdoor Action Conference on Aquifer Restoration, Groundwater Monitoring and Geophysical Methods; 23-26 May, 1988; Las Vegas, NV; Association of Ground Water Scientists and Engineers & US EPA EMSL, Vol 1; pp. 31-51
- Lee, M. in Marbek Resource Consultants, Ltd. (1989); "Aerobic Remediation R&D"; Remediation Technologies for Treating Hydrocarbon Contaminated Groundwater and Soil: AN R, D & D PLAN OF ACTION. A report on meetings held in Collingwood, Ontario 11/28-12/1 1988 and Calgary AL 1/11/89; Waste Water Technology and Industrial Programs Branch, Environment Canada et al.; pp. 17-18
- Lee, K.; Levy, E. M. (1989); "Enhancement of the Natural Biodegradation of Condensate and Crude Oil on Beaches of Atlantic Canada"; presented at the 11th Biennial International Conference on the Prevention, Behavior Control and Cleanup of Oil Spills; San Antonio Convention Center, San Antonio, TX, 13-16 February, 1989
- Lee, M. D.; Thomas, J. M.; Borden, R. C.; Bedient, P. B.; Ward, C. H.; Wilson, J. T. (1988); "Biore Restoration of Aquifers Contaminated with Organic Compounds"; CRC Critical Reviews in Environmental Control, Vol. 18 Issue 1, 1988; pp. 29-89
- Lee, M. D. and Ward, C. H. (1985); "Biological Methods for the Restoration of Contaminated Aquifers"; Environmental Toxicology and Chemistry, V 4; Pergamon Press Ltd.; pp. 743-750



- Loehr, R. C.; Martin, J. H.; Heuhausser, E. F.; Norton, P. A. and Malock, M. R. (1986); "Land Treatment of Oily Waste - Degradation, Immobilization, and Bioaccumulation"; U. S. Environmental Protection Agency; Center for Research and Development/Center for Research Information, Cincinnati, OH, Robert S. Kerr Environmental Research Laboratory, Ada, OK; EPA/000/SZ/65/009
- Maaskant, W.; Lexmond, W.; Pette, D. and van Rhijn, H. X. in Assink, J. W.; Van Den Brink, W. J. (editors) (1986); "Treatment of Contaminated Groundwater: An Overview of Recent Dutch Experiences"; Contaminated Soil; Proceedings of the First International TNO Conference on Contaminated Soil, (11-15 November, 1985, Utrecht, The Netherlands) Marinus Nijhoff Publishers; Dordrecht, The Netherlands; pp. 807-815
- Mackay, D. M.; Cherry, J. A. (1989); "Groundwater contamination: Pump-and-treat remediation"; Environmental Science Technology, Vol. 23, No. 6, 1989; pp. 630-636
- Major, D. W.; Mayfield, C. I. and Barker, J. F. (1988); "Biotransformation of Benzene by Denitrification in Aquifer Sand"; Groundwater, Vol. 26, No. 1, January-February, 1988; pp. 8-13
- Matson, C. (1985); "Considerations for the Current and Future Practice of Bioreclamation of Organic Contaminants/Important Aspects of Field Applicability in Ground Water Restoration"; Proceedings of the Fifth National Symposium on Aquifer Restoration and Ground Water Monitoring held 21-24 May, 1985 Columbus, OH; National Well Water Association, Worthington, OH; pp. 497-504
- Mayfield, C. I. in Marbek Resource Consultants, Ltd. (1989); "Anaerobic Bioremediation R&D"; Remediation Technologies for Treating Hydrocarbon Contaminated Groundwater and Soil: AN R, D & D PLAN OF ACTION. A report on meetings held in Collingwood, Ontario 11/28-12/1 1988 and Calgary AL 1/11/89; Waste Water Technology and Industrial Programs Branch, Environment Canada et al.; pp. 18-19



- McNabb, J. (1989); personal communications during interviews; Robert S. Kerr Research Laboratory, Ada OK; 10 July, 1989
- National Technical Information Service (NTIS), U. S. Department of Commerce (1988a); "Biodeterioration of Oil Spills, January, 1970-January, 1988, (Citations from the NTIS database)"; NTIS No. PB88857594XSP
- National Technical Information Service (NTIS), U. S. Department of Commerce (1988b); "Hazardous Materials: Microbiological Decomposition. January 1978-August 1988 (Citations from the Life Sciences Collection Database)"; NTIS No. PB88867593SXP
- Nunno, T. J.; Hyman, J. A.; Pheiffer, T. (1988); "Assessment of Site Remediation Technologies in European Countries"; Superfund '88, Proceedings of the 9th National Conference, Nov 28-30, 1988, Washington, D.C.; Hazardous Material Control Institute; pp. 193-198
- Offutt, C. H.; Knapp, J. O.; Cord-Duthinh, E.; Bissex, D. A.; Oravetz, A. W. Jr.; Lacy, G. D.; Kenney, P. J.; Green, E. L.; Bhinge, D. (1988); "Analysis of Contaminated Soil Treatment Effectiveness"; Superfund '88, Proceedings of the 9th National Conference, Nov 28-30, 1988, Washington, D.C.; Hazardous Material Control Institute; pp. 429-434
- Raymond, R. L. (1974); Reclamation of Hydrocarbon Contaminated Groundwaters; U. S. Patent 3,846,290; 5 November, 1974
- Raymond, R. L.; Jamison, V. W. and Hudson, J. O. (1976); "Beneficial Stimulation of Bacterial Activity in Groundwaters Containing Petroleum Products"; Water-1976: I. Physical, Chemical Waste Water Treatment; AIChE Symposium Series V 166, v 73; pp. 390-404
- Raymond, R. L.; Jamison, J. O.; Mitchell, R. E.; Farmer, V. E. (1978); Field Application of Subsurface biodegradation of gasoline in a Sand formation; Suntech, Inc and Exxon Co.; Project No. 307-77; American Petroleum Institute Publication (API) No. 4430



- Reinhard, M.; Goodman, N. L. and Barker, J. F. (1984); "Occurrence and distribution of organic chemicals in two landfill leachate plumes."; Environmental Science and Technology, Vol. 18, No. 12, December, 1984; pp. 953-961
- Rifai, H. S.; Bedient, P. B.; Wilson, J. T.; Miller, K. M.; Armstrong, J. M. (1988); "Biodegradation Modeling at Aviation Fuel Spill Site"; Journal of Environmental Engineering October, 1988 Vol. 114, No. 5; American Society of Chemical Engineers; pp. 1007-1029
- Ross, D.; Maziarz, T. P.; Bourquin, A. W. (1988); "Bioremediation of Hazardous Waste Sites in the USA: Case Histories"; Superfund '88, Proceedings of the 9th National Conference, Nov 28-30, 1988, Washington, D.C.; Hazardous Material Control Institute; pp. 395-397
- Ruddiger, G. (1987); "Biological *In Situ* Clean up of Oil Contaminations - Yes or No?"; Erd-ol Erdgas Kohle V 103, No. 7-8; July/August 1987; pp. 334-336
- Schmokel, N. (1989); personal communications during project reports to Navy Petroleum Office, Cameron Station, Alexandria, VA; July-August, 1989
- Shepard, M. (1989); "Amoco will employ army of bacteria to eat sludge at Sugar Creek refinery"; Kansas City Star, 14 July, 1989; pp. A-1,A-4
- Sims, R. C.; Sims, J. L.; Sorensen, D. L.; Hastings, L. L.; Doucette, W. J. (1986); "Waste/Soil Treatability Studies for Four Complex Industrial Wastes: Methodologies and Results. Volume 1. Literature Assessment, Waste/Soil Characterization, Loading Rate Selection. Final Report"; U. S. Environmental Protection Agency, Robert S. Kerr Environmental Research Lab, ADA, OK
- Skladany, G. (1988); "Onsite Biological Treatment of an Industrial Landfill Leachate: Microbiological and Engineering Considerations"; paper presented to the First Annual Conference on Genetically Engineered or Adapted Microorganisms in Hazardous Waste Treatment; Washington, D.C., December 1, 1988







- Skladany, G. J.; Sullivan, K. M. (1987); "'Decay Theory' Biological Treatment for Low-level Organic Contaminated Groundwater and Industrial Waste"; Proceedings of the 8th National Superfund Conference; Washington, DC, Nov, 1987; Hazardous Material Control Research Institute
- Skladany, G. J.; Thomas, J. M; Fisher, G.; Ramachadran, R. in Bell, J. M. (editor) (1987); "The Design, Economics, and Operation of a Biological Treatment System for Ketone Contaminated Ground and Solvent Recovery Process Waters"; Proceedings of the 42nd Purdue University Industrial Waste Conference, 12-14 May, 1987; Purdue University, West Lafayette, Indiana; Lewis Publishers, Chelsea, MI; pp. 53-58
- Smith, J. (1989); personal communication during interview 21 July, 1989 at Region VII Headquarters of the U. S. Environmental Protection Agency, Kansas City, KS
- Smith, R. J.; Collins, A. G. (1984); State of the Art of Microbial Enhanced Oil Recovery: a Review of the Literature, NIPER-12(DE84017403); Office of Scientific and Technical Information, U. S. Department of Energy, Bartlesville, OK
- Spain, J. C.; Milligan, J. D.; Downey, D. C. and Slaughter, J. K. (1989); "Excessive Bacterial Decomposition of H<sub>2</sub>O<sub>2</sub> During Enhanced Biodegradation"; Ground Water, Vol. 27, No. 2, March-April, 1989; pp. 163-167
- Stetzenbach, L. D. A. (1986); "The Degradation and Utilization of Polycyclic Aromatic Hydrocarbons by Indigenous Soil Bacteria; Phd Thesis; Arizona State University, Tucson, AZ
- Stover, E. (1989); "Coproducted Ground Water Treatment and Disposal Options During Hydrocarbon Recovery Operations"; Ground Water Management Review Winter, 1989; pp. 75-82



- Sveum, P.; Ladousse, A. (1989); "Biodegradation of Oil in the Arctic: Enhancement by Oil-Soluble Fertilizer Application" presented at the 11th Biennial International Conference on the Prevention, Behavior Control and Cleanup of Oil Spills; San Antonio Convention Center, San Antonio, TX, 13-16 February, 1989
- Swindoll, C.M. (1988); "Biodegradation of JP-5 Aviation Fuel by Subsurface Microbial Communities, Progress report 1 January - 15 March 1987"; Naval Civil Engineering Laboratory, Port Hueneme, CA
- Thomas, J. M.; Lee, M. D.; Bedient, P. B.; Borden, R. C.; Canter, L. W. (1987a); "Leaking Underground Storage Tanks: Remediation with Emphasis on 'In situ' Biore Restoration."; U. S. Environmental Protection Agency, Robert S. Kerr Environmental Research Lab, Ada, OK (EPA600287008)
- Thomas, J. M.; Lee, M. D. and Ward, C. H. (1987b); "Use of Ground Water in Assessment of Biodegradation Potential in the Subsurface"; Environmental Toxicology and Chemistry Vol. 6; pp. 607-614
- Thomas, J. M.; Ward, C. H. (1989); "In situ biore restoration of organic contaminants in the subsurface"; Environmental Science and Technology, Vol. 23, No. 7; pp. 760-766
- Torpy, M. F.; Stroo, H. F. and Brubaker, G. (1989); "Biological treatment of hazardous waste"; Pollution Engineering; Vol. 21, No. 5, May, 1989; pp. 80-86
- Trickett, J. F. (1989); personal communication during telephone interview; Regional Environmental, Safety and Regulatory Affairs Manager, AMOCO Inc, Houston, TX, 25 July, 1989
- Vandegrift, S. A. and Kampbell, D. H. (1987); "Gas Chromatographic Determination of Aviation Gasoline and JP-4 Jet Fuel in Subsurface Core Samples"; Journal of Chromatographic Science Vol 26; pp. 566-569



- Wetzel, R. S.; Durst, C. M.; Davidson, D. H.; Sarno, D. J. (1987); "In Situ Biological Treatment test at Kelly Air Force Base, Volume II: Field Test Results and Cost Model"; U. S. Air Force Engineering & Services Center; Tyndall AFB, FL
- Whitfill, D. L. and Boyd, P. A. (1987); "Soil Farming of Oil and Mud Drill Cuttings"; Proceedings, International Association of Drilling Contractors/Society of Petroleum Engineers Drilling Conference; New Orleans, LA, March 15-18, 1987; pp. 429-438
- Wilson, B. H.; Bledsoe, B. and Kampbell, D. in Averett, R. C. and McKnight, D. M. (1986a); "Biological processes occurring at an aviation gasoline spill site"; CHEMICAL QUALITY of WATER and the HYDROLOGIC CYCLE, Proceedings of the Chemical Quality of Water and the Hydrologic Cycle Symposium held at the 8th Rocky Mountain Regional Meeting of the American Chemical Society, Denver CO, 8-12 June, 1986; Lewis Publishers Inc; Chelsea, Mi; pp. 125-137
- Wilson, B. H.; Bledsoe, B. E.; Kampbell, D. H.; Wilson, J. T.; Armstrong, J. M.; Sammons, J. H. (1986b); "Biological Fate of Hydrocarbons at an Aviation Gasoline Spill Site"; presented at the Prevention Detection and Restoration Conference and Exposition, Houston TX, November 12-14, 1986; jointly sponsored by the National Water Well Association and the American Petroleum Institute
- Wilson, B. H.; Smith, G. B.; Rees, J. F. (1986c); "Biotransformations of Selected Alkylbenzenes and Halogenated Aliphatic Hydrocarbons in Methanogenic Aquifer Material: a Microcosm Study"; Environmental Science and Technology, Vol. 20, No. 10; pp. 997-1002
- Wilson, J. T.; Henson, J. M.; Piwoni, M. D.; Wilson, B. H.; Bannerjee, P. (1988); "Biodegradation and Sorption of Organic Solvents and Hydrocarbon Fuel Constituents in Subsurface Environments" ESL-TR-87-52; Engineering & Services Laboratory, Air Force Engineering & Services Center, Tyndall Air Force Base, Florida



- Wilson, J. T.; Leach, L. E.; Henson, M.; Jones, J. N. (1986); "In situ Bio restoration as a Ground Water Remediation Technique"; Ground Water Monitoring Review, Fall, 1986; pp. 56-64
- Wilson, J. T. and Ward, C. H. in Pierce, G. (editor) (1987); "Opportunities for bioreclamation of aquifers contaminated with petroleum hydrocarbons"; Developments in Industrial Microbiology, (Journal of Industrial Microbiology, Suppl. No 1), Vol 27; Society for Industrial Microbiology; pp. 109-116
- Yaniga, P. M.; Matson, C.; Demko, D. J. (1985); "Restoration of Water Quality in a Multiaquifer System via In Situ Biodegradation of the Organic Contaminants"; Proceedings of the Fifth National Symposium on Aquifer Restoration and Ground Water Monitoring held 21-24 May, 1985 Columbus, OH; National Well Water Association, Worthington, OH; pp. 510-526
- Zeyer, J.; Kuhn, E. P. and Schwarzenbach, R. P. (1986); "Rapid Microbial Mineralization of Toluene and 1,3-Dimethylbenzene in the Absence of Molecular Oxygen"; Applied and Environmental Microbiology V 52, n 4; American Society for Microbiology; pp. 944-947





# SUGAR CREEK REFINERY SLUDGE TREATMENT DATA

<u>Component</u>	Composition of untreated sludge (Average)	Composition of sludge residue after 2-3 months LST treatment (Average)
Oil and Grease (% by wt.)	39.4	20.6
Total Solids (% by wt.)	43.65	42.21

## Organics (ppb)

Naphthalene	222,500	est. < 10,000
Acenaphthylene	25,000	none detected
Acenaphthene	40,000	est. < 10,000
Fluorene	152,500	10,100
Phenanthrene	355,000	32,800
Anthracene	55,000	12,400
Fluoranthene	60,000	est. < 10,000
Pyrene	132,500	96,600
Benzo (a) anthracene*	107,500	57,100
Chrysene*	157,500	86,300
Benzo (b) fluoranthene*	45,000	est. < 12,300
Benzo (k) fluoranthene*	27,500	est. < 10,000
Benzo (a) pyrene*	67,500	52,600
Indeno (1,2,3-cd) pyrene*	25,000	none detected
Dibenzo (a,h) anthracene*	25,000	none detected
Benzo (g,h,i) perylene*	30,000	none detected

\*Potentially carcinogenic PAHs

## Inorganics (ppm)

Antimony	not reported	est. 2
Arsenic	36.5	12.03
Barium	not reported	36.27
Beryllium	not reported	.126
Cadmium	1.6	.629
Chromium	1,324	1,226
Cobalt	not reported	3.63
Lead	1,500	437
Mercury	not reported	.25
Nickel	245	50.5
Selenium	not reported	.636
Vanadium	not reported	28.4

Appendix A











Thesis

L884

Lubbers

c.1

Biodegradation of hydrocarbons as a remediation method for petroleum contaminants in the environment or as a treatment method for petroleum wastes.

Thesis

1,884

Lubbers

c.1

Biodegradation of hydrocarbons as a remediation method for petroleum contaminants in the environment or as a treatment method for petroleum wastes.





thesL884

Biodegradation of hydrocarbons as a reme



3 2768 000 86848 3

DUDLEY KNOX LIBRARY